ADDIS ABABA UNIVERSITY

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BOTANICAL SCIENCE STREAM

SURVEY AND SEROLOGICAL DETECTION OF SWEET POTATO (*IPOMOEA BATATAS* (L.) LAM.)INFECTING VIRUSES IN ETHIOPIA

A Thesis Submitted to School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science in Biology

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June, 2010

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Declaration

I, the undersigned, declare that this thesis is my original work. It has never been presented for a degree in any other institution and that all sources of materials used in it have been duly acknowledged.

Name: _____

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Date: _____

This thesis has been submitted for examination with my approval as University advisor.

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Tileye Feyissa (PhD)

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List of Abbreviations

AARC-Awassa Agricultural Research Center

AHRC-Holetta Agricultural Research Center

BCIP-5-Bromo-4-Chloro-3'-Indolyphosphate

cDNA- Complimentary Deoxyribonucleic Acid

CIP-International Potato Center

DAS-ELISA-Double Antibody Sandwich Immunosorbent Assay

DMF- Dimethylformamide

DR-Congo- Democratic Republic Congo

EIAR- Ethiopian Institute of Agricultural Research

FAO-Food and Agriculture Organization

GAR-Goat Anti-Rabbit

IgG- Immunoglobulin G

mAb- Monoclonal Antibody

NBT- Nitro-Blue Tetrazolium Chloride

NCM-ELISA-Nitrocellulose Membrane Enzyme-linked Immunosorbent Assay

pAb-Polyclonal Antibody

PBS- Phosphate Buffer Saline

PBS-T- Phosphate Buffer Saline+Tween20

PCR-Polymerase Chain Reaction

PNG- Papua New Guinea

PVP- Polyvinyl pyrolidine

RAM-ap-Anti Mouse-Alkaline Phosphatase Conjugate

TAS-ELISA- Triple Antibody Sandwich Enzyme-linked Immunosorbent Assay

TBS-Tris-Buffer Saline

T-TBS- Tris-Buffer Saline-Tween20

List of Virus Acronyms and Names

SPFMV- *Sweet potato feathery mottle*

SPCSV- *Sweet potato chlorotic stunt virus*

SPVG- *Sweet potato virus G*

SPMMV- *Sweet potato mild mottle virus*

SPCFV- *Sweet potato chlorotic fleck virus*

SPLV- *Sweet potato latent virus*

SPCaLV- *Sweet potato caulimo-like virus*

CMV- *Cucumber mosaic virus*

SPLCV- *Sweet potato leaf curl virus*

SPLCV- *Sweet potato leaf curl virus*

SPLSV -*Sweet potato leaf speckling luteovirus*

SPRSV -*Sweet potato ring spot nepovirus*

SPYDV -*Sweet potato yellow dwarf ipomovirus*

SPVMV -*Sweet potato vein mosaic potyvirus*

SPLCV -*Sweet potato leaf curl badnavirus*

SPLCV-US- *Sweet potato leaf curl geminivirus*

SPCLCV-I*pomoea crinkle leaf curl geminivirus*

SPVD-*Sweet potato virus disease*

SPCDD-*Sweet potato chlorotic dwarf disease*

Abstract

The current study was conducted to assess the recent magnitude of virus diseases attacking sweet potato in the chief production areas of country. Thus, a total of 235 symptomatic and 735 asymptomatic sweet potato vines were collected. Samples from farmers' fields were established in an insect-proof screen house and were tested for viruses by nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) together with 624 imported in vitro plantlets. During the field survey it was observed that incidences of virus and virus-like symptom were high in Sidama, Wolayita, Awassa and Hadiya and very low in Gamo-Gofa and Kembata-Tembaro. The viruses detected were sweet potato feathery mottle virus (SPFMV), sweet potato chlorotic stunt virus (SPCSV) and sweet potato virus G (SPVG). The most prevalent virus detected was SPFMV. The second most frequent virus was SPCSV and SPVG being the third yet, low frequent virus. From imported in vitro plantlets 19.2% was reacted positive for Anti-SPFMV and only 0.2% sero-positive for Anti-SPCSV. SPVD was the most common co-infection observed followed by SPVG+SPCSV yet, less frequent. None of the samples obtained from Eastern and Western Hrarge was any virus infection detected. TAS-ELISA was performed for weakly reacted samples and 35.7% was reacted positive for mAb of SPFMV. However, none of the samples obtained from East and West Hararge was sero-positive for mAb of SPFMV. This study has provided a quantitative assessment of both single and co-infection of viruses in sweet potato plants in Ethiopia, and elucidated the importance of developing resistant varieties particularly against the detected sweet potato viruses, production of virus free materials and quarantine measures not to be overlooked specially in high SPVD incident areas.

Key words/Phrases: Sweet Potato, NCM-ELISA, TAS-ELISA, Incidence, Virus, Mixed Infection, Single Infection

1. Introduction

Sweet potato, *Ipomoea batatas* (L.) Lam. is a dicotyledonous plant which belongs to the family *Convolvulaceae*. In the family there are approximately 50 genera and more than 1000 species and the genus *Ipomoea* comprises 400 species but, only *I. batatas* is starchy, sweet tasting and an important tuberous root crop. In addition the young leaves and shoots are also sometimes eaten as greens. Sweet potato is distantly related to the potato (*Solanum tuberosum*). It is commonly called a yam in parts of North America, although it is only very distantly related to the widely identified as yams which belongs to the family *Dioscoreaceae*, which is native to Africa and Asia (Sebsebe Demissew, 2006; Vincent, 2009).

Sweet potatoes are native to the tropical parts of the South America, and were domesticated there at least 5000 years ago. They spread very early throughout the region, including the Caribbean. They were also known before western exploration in Polynesia. How they arrived there is a subject of ongoing research and discussion of various hypotheses involving archaeological, linguistic and genetic evidence. There is an assumption that the centre of origin of *I. batatas* was to be between the Yucatán Peninsula of Mexico and the mouth of the Orinoco River in Venezuela (Nishiyama, 1971; Haberle, 1998).

The 'cultigens' had mostly likely been spread by local people to South America by 2500 BC provided strong supporting evidence that the geographical zone postulated by Austin is the primary centre of diversity. The much lower molecular diversity found in Peru-Ecuador suggests that this region be considered as secondary centre of sweet potato diversity. Sweet potatoes are now cultivated throughout tropical and warm temperate regions wherever there is sufficient water to support their growth (Austin, 1988).

World production in 2004 was 127,000,000 tons. The majority comes from China with a production of 105,000,000 tons from 49,000 km². About half of the Chinese crop is used for livestock feed. Per-capita production is greatest in countries where sweet potatoes are a staple of human consumption. Today, it is still very popular, although less popular than regular potatoes (Luisa and Robert, 2000; Kapinga *et al*., 2007; Vincent, 2009).

Plant pathogens such as fungus, viruses and bacteria are responsible for increasing economic losses worldwide. Productivity of sweet potato is also greatly constrained by pests and diseases with the

most important one being viral diseases because sweet potato is very sensitive to virus infection. Since 1946-1950's viral diseases have been recognized in quality and yield deterioration of sweet potato in South Africa. However, nowadays sweet potato production is greatly constrained by disease that even may lead to 98% yield reduction (Moyer and Salazar, 1989; Kapinga *et al*., 2007).

A suspected virus disease of sweet potato was reported in 1944 in southern Carolina but 20 viruses have been recently reported to infect sweet potato (Fuglie, 2007). These include the *sweet potato feathery mottle virus* (SPFMV), *sweet potato chlorotic stunt virus* (SPCSV), *sweet potato virus G* (SPVG), *sweet potato mild mottle virus* (SPMMV), *sweet potato chlorotic fleck virus* (SPCFV), *sweet potato latent virus* (SPLV), *sweet potato caulimo-like virus* (SPCaLV), *cucumber mosaic virus* (CMV) and *sweet potato leaf curl virus* (SPLCV). Viruses often occur in multiple infections in the field with the most commonly encountered combination being that between SPFMV and SPCSV. This dual infection is responsible for the severe sweet potato virus disease (SPVD) which has been indicated to be the major viral disease in East Africa. Recently synergetic combination of three viruses of sweet potato namely SPFMV, SPCSV and SPMMV cause sweet potato chlorotic dwarf disease (SPCDD) found in Argentina (Chavi *et al*., 1997; Mukasa *et al*., 2003).

Detection and characterization of sweet potato viruses is crucial in the understanding of the epidemiology of the disease(s) caused by these viruses, development of infectivity-based forecasting systems and control strategies. Study of several of these diseases has been hampered by lack of simple detection techniques. Though sweet potato viruses have been detected by observing symptom expression, host range studies and some by their vector relationship, the primary tests to detect sweet potato viruses are bioassays on indicator plants by observing symptoms, vector transmission procedures and serology using enzyme-linked immunosorbent assay (ELISA) and molecular methods using polymerase chain reaction (PCR) methods (Chavi *et al.,* 1997; Moyer and Salazar, 1989).

Viruses have previously never been major limiting factors in sweet potato production in Ethiopia. The first report of a virus on sweet potato in the country was made over two decades ago by electron microscopy of sweet potato plants with mosaic symptoms from Nazreth, the virus being tentatively identified as SPFMV (SPL, 1986). In addition *sweet potato virus 2* (SPV2) was also reported in research stations in Ethiopia Recently, however, Tameru Alemu, (2004) reported a high incidence of SPFMV in some fields and the occurrence of another virus named as SPVG mainly

from Wolayta zone. In the current survey, three viruses namely *sweet potato feathery mottle virus* (SPFMV), *sweet potato chlorotic stunt virus* (SPCSV), *sweet potato virus G* (SPVG) were reported.

2. Literature Review

2.1. Taxonomy

Convolvulaceae is a family of herbaceous and woody, often climbing species, which is well distributed throughout temperate and tropical latitudes in a wide range of habitats. Many species have long, trailing stems and are typical of rich vegetation, or open drier places, including sand dunes. The woody species are characteristic of tropical regions and, in open woodland, large trees up to 10 m high can occur. Species in the family *Convolvulaceae* have alternate and simple leaves; flowers are bisexual with five free sepals, five fused petals and five stamens fused at the base of the corolla tube; fruit is dehiscent capsule (Sebsebe Demissew, 2006).

Ovary, styles and stigmas are also used for identification purpose in the group. In the family *convolvulaceae* there are approximately 50 genera and more than 1000 species and *Ipomoea* is a large genus composed of approximately 400 species. Most of them are annual and perennial herbaceous vines, with a few erect shrubs found in the tropics. A number of African and Australian species are collected from the wild as emergency foods. In South-east Asia and Melanesia, *I. batatas* is cultivated for its delicious root tuber (Jarret *et al*., 1992; Haberle, 1998).

2.2. Morphological Description of *Ipomoea batatas* (L.) Lam.

Ipomoea batatas is a vine-like, perennial herb, treated as an annual when cultivated. It has trailing or twining stems containing latex which exudes when cut. The stems can be prostrate or ascending, often twining, glabrous or pubescent and light green to purple in color. The long, thin stems that creep on the surface produce extensive, fibrous roots where nodes make contact with the soil; tuber flesh color may be white, yellow, orange, reddish or purple. Stem length ranges from 1 to 5 m, depending on genotype (Sebsebe Demissew, 2006).

Leaves shape are highly variable, sometimes on the same plant, depending on their age. They are arranged spirally and are simple and estipulate, with petioles measuring between 5 and 30 cm long. Their laminas are mostly ovate and can be entire to deeply digitately lobed, with their base usually cordate. Their tips can be acute or obtuse and the leaves can be glabrous, or with variable pubescence. Their color is also highly variable, from light green to deep purple, sometimes with purple stain at their base, or with green or purple veins beneath. Flowers are solitary or in clusters of buds, flower has five sepals, five petals, five stamens, a compound pistil, fused corolla and

whose colors vary pink to deep purple. Five stamens are attached at the base of the corolla and are of variable length. The ovary is two-celled; contain up to four seeds, but usually only one or two are fully developed (Jarret *et al*., 1992; Huaman, 1996; Sebsebe Demissew, 2006).

2.3. History of Origin and Domestication

Sweet potato originated on the American continent. Based on the number of related species and analysis of their morphological variation, the geographical centre of origin of *Ipomoea batatas* and its wild relatives has been thought to be between the Yucatan peninsula in Mexico and the Orinoco River in Venezuela (Nishiyama, 1971; Yen, 1982; Haberle, 1998). The much lower molecular diversity found in Peru-Ecuador suggests that this region be considered as secondary centre of sweet potato diversity. It seems that the oldest remains of dried sweet potatoes are from the caves of the Chilca Canyon in Peru, which have been radiocarbon dated to 8000 years old (Rossel and Zjang, 2001).

Sweet potato roots have also been excavated from an archaeological site located in the Casma Valley of coastal Peru, dated between 1785 and 1120 BC. An analysis of their starch granules revealed that they were significantly smaller in size compared to the modern cultivars but that they were definitely from the species *I. batatas*. Morphological analysis of the various related species indicates that *I. trifida* is the closest wild relative to the sweet potato but *I. tabascana* is also morphologically very close (Austin, 1987, 1988; Huang *et al*., 2000; Montenegro *et al*., 2007).

2.4. Global Distribution

Sweet potato is now cultivated in more than 100 developing countries. It is typically a smallholder farmer crop grown on marginal soils with limited inputs. In these countries, yields are well below the average of developed countries. The sweet potato is a dry-land crop tolerant of a wide range of edaphic and climatic conditions. It is more tolerant to cold than other tropical root and tuber crops and therefore it can be grown at altitudes as high as 2500 m (Luisa and Robert, 2000).

It is also an important global food crop in terms of both area and production. However, sweet potato distribution is extremely concentrated, yet sweet potato production is spread over many countries. Vietnam, Indonesia, India, and the Philippines are other important sweet potato- producing countries in Asia. There is a conspicuous concentration of sweet potato in the East African Highlands (Ethiopia, Uganda, Rwanda, Burundi, and Kenya), Papua New Guinea and in other parts

of the world where, sweet potato has an important role in the diet. Between the early 1960s and late 1990s, there was a global reduction in sweet potato area of about 31%. Due to increases in yield, the associated drop in production is less, especially in China (La Bonte and Cannon, 1998; Luisa and Robert, 2000; Adane Abraham, 2007).

2.5. Importance of Sweet Potato

Sweet potato with a mean annual production of 133 million tons from 1991-2000 is ranked among the top ten most important food crops globally. Thus, it is the world's seventh most important food crop after wheat, rice, maize potato, barley, and cassava and the leading root crop grown more in developing countries. World sweet potato production is around 133 million tons in an area of 9.2 million hectare. Sweet potatoes are grown in over 100 countries and over 98% of their production is consumed in developing countries. For every calorie consumed sweet potatoes provide over 90% of essential nutrients except for protein and niacin. Orange-fleshed sweet potatoes are particularly nutritious, ranking highest in nutrient content of all vegetables for vitamins A and C, iron, copper, calcium, and fiber (Zhang and Li, 2004; Kapinga *et al*., 2007).

Both the amount of sweet potato consumed and the manner in which it is consumed vary widely. Sweet potato consumption varies within countries, by regions, by time of year, and by income group. Green tips are used as a vegetable in some areas and can be an important source of protein and micronutrients. Sweet potato is widely used by small farmers to sustain local livestock production systems (Luisa and Robert, 2000; Assefa Tofu *et al.*, 2007). Sweet potato roots and vines are being increasingly used in pig and other livestock systems in China, and FAO estimated that 50 million tons in 2002 was used as feed. About 45% of Asia's domestic sweet potato supply is used for animal feed, and nearly 50% is used for human consumption, either as fresh or processed products (Zhang and Li, 2004). In contrast, 85% of Africa's domestic sweet potato supply is for human consumption. For example, per capita sweet potato consumption in Rwanda is estimated as 147 kg/ year; Burundi, 120 kg/year; and Uganda, 88 kg/ year, and most of this occurs in the drier seasons (Kapinga *et al*., 2007).

Sweet potato can be used as a seasonal staple when there is a shortage of other foodstuffs. It is also an important crop in northern regions of South Africa. In Africa, commercial farmers use vines as fodder for livestock. Humans consume both the roots and young leaves and tips as green vegetables. Sweet potato root is generally consumed boiled (and mashed) or fried. It is also

processed commercially or at home into flour that is then often mixed with other flours to make composite flour. These flours are used to make porridge, which is an important complementary food for breastfed children. Less commonly, the flour is used to make bread and pastries. Poor households tend to eat boiled or baked sweet potato roots (Ewell and Mutuura, 1991).

Fresh roots are also fried and the chips eaten as a snack food. In China, humans mostly consume sweet potato as a vegetable or health food, not as a staple food, and the roots are typically boiled or baked. Sweet potato starch is used for making fresh or dry starch noodles and sheets, and for fresh starch cakes. Snacks of dry sweet potato slices and bars are also popular. In Japan, consumers use many of the deeply colored root varieties and consume sweet potato mostly as processed snacks, noodles, and candies (Zhang and Li, 2004; Fuglie, 2007).

Sweet potato is grown and the roots are consumed in the drier and poorer regions of India. In Latin America, there is great diversity of varieties. Most sweet potato roots are again eaten boiled or baked. Sweet potato is the main staple in some South Pacific islands, especially in Papua New Guinea highlands where many varieties are available, providing the dominant portion of the energy and protein requirements of the population. In Oceania, in 2002, it was the most important tuber and root crop after potato. For long-term storage, the roots are sliced into thin chips for food or strips for animal feed that are sun or shade-dried. Chips can be stored in the dried state, rehydrated, and then cooked. Alternatively, they can be ground into flour that can be mixed with wheat as composite flour; the proportions of wheat and sweet potato vary depending on taste. Pure or composite flour is used to make gruels, bread, donuts, cakes, and other products (La Bonte and Cannon, 1998).

Starch is extracted and used to make noodles, sheets, and cakes in China. In some countries, processed products made from sweet potato, including starch, noodles, candy, desserts, and flour, are made by farm households to extend the availability, diversify the use of, and increase the value of the crop. In China, in particular, production of sweet potato starch in recent years has evolved into a cottage industry that uses millions of tons of roots per year as raw material inputs. The magnitude of these new uses is not easy to quantify in a systematic way; partly for that reason, the available statistics on processing do not always reflect their true level of importance. Sweet potato is also consumed in Europe and the United States as a cooked vegetable. Sweet potato has high carbohydrate content (La Bonte and Cannon, 1998; Zhang and Li, 2004).

2.6. Sweet Potato Production

World production in 2004 was 127,000,000 tons. The majority comes from China with a production of 105,000,000 tons from 49,000 km². In the U.S., North Carolina, the leading state in sweet potato production, provided 38.5% of the 2007 U.S. production of sweet potatoes. In 2007, California produced 23%, Louisiana 15.9%, and Mississippi 19% of the U.S. total. Sweet potato became a favorite food item of the French and Spanish settlers and thus continued a long history of cultivation in Louisiana (La Bonte and Cannon, 1998; Luisa and Robert, 2000; Vincent, 2009). Mississippi is also a major sweet potato producing state; with about 150 farmers are growing sweet potatoes on approximately 820,033 km^2 and contributing $19 million dollars to the state's economy.

China is the largest grower of sweet potatoes; providing about 80% of the world's supply, 130 million tons were produced in one year. Historically, most of China's sweet potatoes were grown for human consumption, but now most (60%) are grown to feed pigs. The rest are grown for human food and for other products. Some are grown for export, mainly to Japan. China grows over 100 varieties of sweet potato (Zhang and Li, 2004).

Ethiopia has 73 million people and is second most populous country in Africa. Sweet potato is grown around a densely populated area of south, southwestern and eastern part of the country and is one of the most important crops for at least 20 million Ethiopians. The total area under sweet potato in Ethiopia is 75000ha with an average productivity 8t/ha (Van Bruggen, 1984; Assefa Tofu *et al.*, 2007; U.S Cenesus Bureau, 2009).

2.7. Production Constraints of Sweet Potato

Plant pathogens caused by fungus, viruses and bacteria are responsible for economic losses worldwide. They can cause a large range of symptoms in most cultivated plants, which can be affected in their different parts with various agronomic impacts. Productivity of sweet potato is greatly constrained by pests and diseases the most important one being viral diseases because it is very sensitive to virus infection. Depending on cultivar, infecting virus, stage of infection and whether the crop is infected with a single or multiple viruses, viral diseases may cause up to 100% yield loss (Gibson *et al*., 1997).

Since 1946-1950's viral diseases have been recognized in quality and yield deterioration of sweet potato in South Africa. Production of sweet potato is greatly constrained by disease that cause yield

reduction by 98% and yield also severely limited in the tropics. Africa countries such as Nigeria and Uganda it accounted for 50% yield loss. In East Africa, over 90% yield reductions have been associated with viruses. Since, sweet potato is vegetatively propagated crop; viruses are spread from mother plants to new plants during the propagation cycle (Cohen *et al.,* 1997; Gibson *et al*., 1998).

3. Classification Bases of Plant Viruses and Etiology of Sweet Potato Viruses

Viruses are very small (submicroscopic) infectious particles (virions) composed of a protein coat and a nucleic acid core. They carry genetic information encoded in their nucleic acid, which typically specifies two or more proteins. Translation of the genome (to produce proteins) or transcription and replication (to produce more nucleic acid) takes place within the host cell and uses some of the host's biochemical "machinery". Viruses do not capture or store free energy and are not functionally active outside their host. They are therefore parasites (and usually pathogens) but are not usually regarded as genuine microorganisms (George, 2005).

Most viruses are restricted to a particular type of host. Some infect bacteria, and are known as bacteriophages, whereas others are known that infect algae, protozoa, fungi (mycoviruses), invertebrates, vertebrates or vascular plants. However, some viruses that are transmitted between vertebrate or plant hosts by feeding insects (vectors) can replicate within both their host and their vector. This web site is mostly concerned with those viruses that infect plants but we also provide some taxonomic and genome information about viruses of fungi, protozoa, vertebrates and invertebrates where these are related to plant viruses (Dube, 2004).

We also provide information about viroids, which are infectious RNA molecules that cause diseases in various plants. Their genomes are much smaller than those of viruses (up to 400 nucleotides of circular single-stranded RNA) and do not code for any proteins (Dube, 2004; George, 2005).

All viruses belong to the kingdom Viruses. Within the kingdom, viruses are distinguished as RNA viruses and DNA viruses, depending on whether the nucleic acid of the virus is RNA or DNA. Viruses are further subdivided depending on whether they possess one or two strands of RNA or DNA of either positive or negative sense, either filamentous or isometric. Within each of these groups there may be viruses replicating *via* a polymerase enzyme (+RNA or DNA viruses) or *via* a

reverse transcriptase (-RNA or DNA viruses). Most viruses consist of nucleic acid surrounded by coat protein, but some also have a membrane attached to them (Cipriani *et al*., 2000; George, 2005).

Some viruses have all their genome in one particle (monopartite viruses), but the genome of other (multipartite) viruses is divided among two, three, or, rarely, four particles. Other characteristics in the classification of viruses include the symmetry of helix in the helical viruses, or number and arrangement of protein subunits in the isometric viruses, size of the virus, and, finally, any other physical, chemical, or biological properties. Etiology refers to the studies of diseases and the factors underlying their spread. Sweet potato infecting viruses are belong to the families: *Bromoviridae* (CMV), *Closteroviridae* (SPCSV), *Potyviridae* (SPFMV, SPLV, SPVG, SPMMV, SPV2 and SPMSV), *Caulimoviridae* (SPCaLV) and *Flexiviridae* (SPCFV) (Fauquet and Mayo, 1991; Di Feo *et al.,* 2000).

3.1. Viral Diseases of Sweet Potato

A suspected virus disease of sweet potato was reported in 1944 in southern Carolina but 20 viruses have been recently reported to infect sweet potato (Fuglie, 2007). These include the *sweet potato feathery mottle virus* (SPFMV), *sweet potato chlorotic stunt virus* (SPCSV), *sweet potato virus G* (SPVG), *sweet potato mild mottle virus* (SPMMV), *sweet potato chlorotic fleck virus* (SPCFV), *sweet potato latent virus* (SPLV), *sweet potato caulimo-like virus* (SPCaLV), *cucumber mosaic virus* (CMV) and *sweet potato leaf curl virus* (SPLCV).there are also other viruses reported to infect sweet potato: *sweet potato leaf speckling luteovirus* (SPLSV), *sweet potato latent potyvirus* (SPLV), *sweet potato ring spot nepovirus* (SPRSV), *sweet potato caulimovirus* (SPCaLV), *sweet potato yellow dwarf ipomovirus* (SPYDV), *sweet potato vein mosaic potyvirus* (SPVMV), *sweet potato leaf curl badnavirus* (SPLCV), *sweet potato leaf curl geminivirus-US* (SPLCV-US), *ipomoea crinkle leaf curl geminivirus* (SPCLCV), and *sweet potato phytoreovirus* (Chavi *et al*., 1997; Gibson *et al*., 1997).

3.1.1. Sweet Potato Feathery Mottle Virus (SPFMV)

Sweet potato feathery mottle virus (SPFMV) is the most important and common virus infecting sweet potato and is found wherever sweet potato is grown. Many strains have been identified and it has been referred to as *russet crack virus, sweet potato virus A, sweet potato ring spot virus, sweet potato leaf spot virus and internal cork virus* (Karyeija *et al.,* 1998, 1998). SPFMV is a member of the family *Potyviridae*, genus *Potyvirus*, the largest family of plant viruses. Like other potyviruses,

the virions are elongate, flexuous rods with a monopartite, single-stranded, positive sense RNA molecule with a particle length of 830-850nm (Cohen *et al.,* 1997). It is readily transmitted by aphids in a non-persistent manner through brief feeds of only 20-30 seconds. The virus is not seed-borne, but like many viruses infecting vegetatively propagated plants, it is also disseminated in tubers and vine cuttings. Symptoms on sweet potato leaves appear as faint to distinct, irregular chlorotic spots occasionally bordered by purplish pigmentation (Ames *et al.,* 1997).

Diffuse mottle along the main veins and vein clearing can also be seen on infected leaves. Leaf symptoms vary with cultivar susceptibility, climatic condition, plant age and strain virulence. Some genotypes also exhibit external and internal root symptoms which include external cracking and internal necrosis depending on the cultivar and virus isolate (Karyeija *et al.,* 1998; 2001). SPFMV is mostly restricted to members of the *Ipomoea,* which include *I. nil* L. (Roth), *I. setosa* and sweet potatoes. Single infections of SPFMV have been reported to cause severe symptoms, which are variable in *I. nil,* and *I. Setosa*. Some isolates infect *Chenopodium amaranticolor* Coste & Reyn, C. *quinoa* Willd, or *Nicotiana benthamiana* Gray, but others seem to be restricted to *Ipomoea* species (Thompson and Mynhardt, 1986).

3.1.2 Sweet Potato Chlorotic Stunt Virus (SPCSV)

It was previously known as *sweet potato sunken vein virus* and SPVD-associated closterovirus (Alicai *et al.,* 1999). It is a member of the family *Closteroviridae*, genus *Crinivirus* with a single-stranded positive-stranded RNA genome (Gibson and Aritua, 2002). Early classification was based on particle lengths, long types with particles from 1.200 to 2.000nm and short types with particles from 700 to 800nm (Liu *et al.,* 2000). Karyeija *et al.* (2000) reported that SPCSV remains confined to the phloem and at similar or slightly lower titer in the SPVD-affected plants. SPCSV is transmitted semi-persistently by whitefly, *Bemisia tabaci* Genn. and *Trialeurodes abutilonea* Haldeman, and not by mechanical means. Although SPCSV can infect plants by itself, it has been identified as a component of synergistic complexes with other viruses (Gibson and Aritua, 2002).

Symptoms vary with plant genotypes. Symptoms caused by SPCSV alone are relatively mild in sweet potato and *I. setosa* and plants may become mildly stunted, chlorotic and purpling of leaves can occur (Alicai *et al.,* 1999). Affected plants commonly produce less than half the tuberous root yield of symptomless ones. SPCSV infects sweet potato and *I. setosa* (Gibson *et al.,* 1997, 1998; 1998). It was reported by Cohen *et al.* (1991) that SPCSV was also found to infect Lisianthus

(Eustoma grandiflorum Raf. Shinn.) SPCSV is known to be distributed in Nigeria, Zambia and Tanzania, and it is also found in Kenya and the Caribbean (Ames *et al.,* 1997; Kaitisha and Gibson, 1999).

3.1.3. Sweet Potato Virus Disease (SPVD)

Sweet potato virus disease (SPVD) is a name used to describe plants affected by a range of severe symptoms associated with a dual infection of SPCSV and SPFMV (Karyeija *et al.,* 1998, 2001). The first report of SPVD may have been in the eastern Belgian Congo (DR Congo) in 1939. It is also the most serious disease of sweet potato in Africa, especially in Uganda (Alicai *et al.,* 1999). Co-infection between SPFMV and SPCSV results in the development of SPVD characterized by severe leaf distortion, including narrowing (strap-like), vein clearing and crinkled leaves, chlorosis, discoloration and stunting of plants. SPVD infection caused yield depression of up to 90% in sweet potato cultivars tested in 1986 in Ekona, Cameroon (Ngeve and Boukamp, 1991). Gibson *et al.* (1998) showed that sweet potato infected with this virus-complex produce 2% of the yield of unaffected sweet potato cuttings. The incidence of SPVD was revealed to be higher in fields planted as monocrop than in other cropping patterns. SPVD is reported in East Africa (Karyeija *et al.,* 2000, 2001).

3.1.4. Sweet Potato Virus G (SPVG)

The *sweet potato virus G* (SPVG) belongs to the family *Potyviridae* Viruses; single stranded RNA positive-strand viruses, no DNA stage; *Potyviridae*, genus *Potyvirus* and is transmitted mostly by whiteflies, and especially by *B. tabaci*. This virus may cause only mild symptoms but it has been observed that symptomless plants may still have a considerable reduction in yield (Cohen and Loebestein, 1991; Slazar and Fuentes, 2000; Ishak *et al*., 2003)

4. Sweet Potato Viruses in East Africa

Viruses affecting sweet potato can be perpetuated and transmitted between cropping cycles by the stem cuttings used as planting material. These viruses are transmitted from one plant to another by sap-sucking insects such as aphids and whiteflies. The *sweet potato feathery mottle virus* (SPFMV) and the related *sweet potato virus 2* (SPV-2) are transmitted mostly by aphids. The *sweet potato virus G* (SPVG) and the *sweet potato mild virus* (SPMV) are transmitted mostly by whiteflies, and

especially by *Bemisia tabaci*. These viruses may cause only mild symptoms but it has been observed that symptomless plants may still have a considerable reduction in yield (Ishak *et al*., 2003; Valverde *et al*., 2004).

The *sweet potato feathery mottle virus* (SPFMV), which causes 'internal cork', is found in many countries and many different strains have been identified. The symptoms are influenced by genotype, environment and the strain involved. The symptoms are mainly on older leaves and consist of the classic chlorotic and feather patterns associated with the leaf midrib. The virus also causes root necrosis and cross-sections of affected storage roots show dark brown, cork-like areas in the flesh. Cultivars have been developed in the USA and have shown high tolerance to 'internal cork'. For control, it is necessary to use stem cuttings from disease-free fields (Laurie and Stork, 1997; Kitisha and Gibson, 1999).

The *sweet potato chlorotic stunt virus* (SPCSV) can cause some dwarfing of the plants and purpling or yellowing of the leaves. When the SPCSV (a crinivirus) and the SPFMV both infect a plant, they interact synergistically to cause the sweet potato virus disease (SPVD), a serious constraint to food productivity and security in East Africa. The symptoms include stunting of the plant and small malformed leaves with chlorotic mottle or vein clearing. Plants affected by SPVD usually produce small storage roots and a severe reduction in yield (Alicai *et al*., 1999; Gutierrez *et al*., 2003).The first report of a suspected virus disease of sweet potato in Eastern Africa was in Democratic Republic of Congo in the late 1930s and then in Uganda in the early 1940s. Later, viral diseases in sweet potato were reported in Kenya, Tanzania Rwanda, Burundi, Malawi, and South Africa. Initial studies indicated the occurrence of two viruses, *virus A* and *virus B*, which were aphid and whitefly transmitted respectively (Alicai *et al*., 1999; Kokkinos and Clark, 2006; Ndunguru and Kapinga, 2007).

Currently, four sweet potato viruses have been identified and confirmed to be widely distributed in East Africa. The four viruses include two that belong to family *Potyviridae*: the *potyvirus sweet potato feathery mottle virus* (SPFMV) and the ipomovirus *sweet potato mild mottle virus* (SPMMV); one belongs to the family *Closteroviridae*: *sweet potato chlorotic stunt virus* (SPCSV) and *sweet potato chlorotic fleck virus* (SPCFV) for which the genus *Carlavirus* has been proposed (Gibson and Aritua, 2002; Mukasa *et al*., 2003). From Ethiopia, three sweet potato viruses were also reported namely SPFMV, SPV2 and SPVG (SPL, 1986; Tameru Alemu, 2004; Adane

Abraham, 2007). Aritua *et al*. (1998) also reported the occurrence of *sweet potato caulimo-like virus* (SPCaLV) in one sample collected in Uganda. A recent survey conducted throughout the developing countries to assess the research needs identified as priorities by local scientists revealed that the control of viruses through varietal resistance, quality planting material and crop management was ranked first (Fuglie, 2007).

5. Sweet Potato Virus Detection and Diagnosis

Detection and characterization of sweet potato viruses is crucial in the understanding of the epidemiology of the disease(s) caused by these viruses, development of infectivity-based forecasting systems and control strategies. Study of several of these diseases has been hampered by lack of simple detection techniques. Sweet potato viruses have been detected by observing symptom expression in the field and host range studies and some by their vector relationship. The primary tests to detect sweet potato viruses are bioassays on indicator plants by observing symptoms, vector transmission procedures and serology using enzyme linked irnrnunosorbent assay (ELISA), polymerase chain reaction (PCR) and electron microscopy (Moyer and Salazar, 1989; Chavi *et al.,* 1997).

5. 1. Serological Detection

Enzyme-linked immunosorbent assay (ELISA) has been used many times to detect plant viruses since its introduction in 1976. It is based on the covalent linkage of an enzyme to an antibody, registering the occurrence of an antigen-antibody complex by rapid enzymatic development of a distinctly colored product. Together with bioassay on indicator plants, ELISA is the primary test to detect plant viruses with polyclonal or monoclonal antibodies (Clark and Bar-Joseph, 1984; Converse and Martin, 1990). Due to lack of sensitivity required, ELISA, which detected SPFMV in partially purified and symptomatic leaves of *I. batatas* and *I. incarnata*, was developed and was found to be a faster, convenient and more sensitive method to confirm SPFMV in sweet potato foliages and other *Ipomoea* spp. (Thottapilly and Rossel, 1988).

The most common ELISA methods that are used are double antibody sandwich-ELISA (DAS-ELISA) and indirect ELISA using the antisera specific for each virus (Hammond *et al.,* 1992; Gibson *et al.,* 1998). Nitrocellulose membrane-ELISA (NCM-ELISA) is also used for detecting viruses such as SPFMV and *sweet potato chlorotic fleck virus* (SPCFV) in sweet potato and *I.*

setosa. It produces results consistent to those obtained using triple antibody sandwich-ELISA (TAS-ELISA) (Gibson *et al.,* 1998). Monoclonal (mAbs) and polyclonal (pAbs) antibodies produced against purified sweet potato viruses have been used to detect sweet potato viruses (Karyeija *et al.,* 2001).

Results from naturally infected sweet potato and grafts indicated that SPFMV occurred in leaves of infected plants at concentrations approaching the limits of ELISA. Thus, proper tissue selection and timing of the assay is critical. It is now widely accepted that SPFMV is most reliably detected by ELISA. Advantages of ELISA are that it can detect viruses in small amounts or in low concentrations and speedy reaction, which is why ELISA is important in virus detection (Singh and Barker, 1991).

However, many serological methods such as most types of ELISA are not sensitive enough to detect antigens at low concentrations and those occurring in complexes as in the case of sweet potato in detecting some viruses, especially when done directly since the plant contains unusually large but variable concentrations of a number of substances such as latex, polyphenols, and polysaccharides that interfere with the assays (Abad and Moyer, 1991). Also, cuttings from older vine parts may harbor viruses not present in the apical portion. These reasons may partly explain why some viruses are not documented as existing in East Africa, making it difficult to discern if absence of detection truly reflects absence of the viruses. Furthermore, the high cost of good quality enzymes and their substrates can also prevent the widespread use of ELISA in developing countries (Walkey, 1991).

5. 2. Polymerase Chain Reaction and Hybridization

The genetic basis of the resistance to these viruses has been investigated in Uganda and Peru. Molecular marker (AFLP and RAPD) studies yielded two genetic markers associated with resistance to SPCSV and SPFMV (Colinet, *et al*., 1994). It is thought that in the presence of both of these viruses, additional genes mediate oligogenic-a phenotypic trait produced by two or more genes working together or multigenic horizontal effects in the sweet potato progenies and breeders are now integrating this information in their schemes combining different sources of resistance (Aritua *et al*., 1998; Kapinga *et al*., 2007).

Polymerase Chain Reaction (PCR) is the use of synthetic nucleic acid probes or the *in vitro* amplification of the specific nucleic acid sequences. It involves making multiple copies of a particular sequence in a genome (virus genome) that is then used to identify the presence of a particular disease. Lack of progress in virus identification and classification and due to the frequent occurrence of mixed infections and synergistic complexes in sweet potatoes (Moyer and Salazar, 1989), PCR technology has been used for identifying and characterizing members of the potyviruses infecting sweet potato. This method provides a convenient way of detecting mixed infections and unknown viruses without preliminary separation and purification of the components of the viral complexes (Colinet *et al.,* 1998).

Genus-specific PCR and subsequent molecular analysis of amplified regions thus comprises a powerful method for the rapid identification and differentiation of potyviruses infecting sweet potato and is the most suitable method for viruses which are difficult to purify or which occur in mixed infections (Colinet *et al.,* 1994 , 1994). Specific primers for detecting and differentiating SPFMV (-CHH and -CH2), SPLV, SPMMV and other viruses have been designed from nucleotide sequences of these viruses (Chavi *et al.,* 1997). Antigen detection can be considerably enhanced by coupling serological trapping of viruses with PCR, the so-called immunocapture-PCR. This provides sensitivity, rapidness and the ability to assay many samples (Mumford *et al.,* 1994).

Hybridization is performed by extracting DNA or RNA from the samples, base denaturing in NaOH, and directly blotting onto nylon membranes. Membranes are then fixed with heat or ultraviolet (UV) radiation and then hybridized with a labeled probe consisting of cDNA sequence of a particular virus (Cohen *et al.,* 1997; Colinet *et al.,* 1994). If the virus is present, the probe will hybridize with its DNA extracts from the sample and if not, the probe is lost during the washing process of the membranes (Lotrakul *et al.,* 1998).

6. Management of Sweet potato Viruses

Managing viruses infecting sweet potato will require knowledge on etiology and ecology of the viruses. Information on the control of viruses and how viruses infect plants is lacking among resource-poor farmers in Africa. Three main control practices are used by African farmers to limit the effects of SPFMV and these are (a) selection of SPVD-resistant cultivars, (b) use of disease free planting material and (c) removing all infected plants (Karyeija *et al.,* 1998). Efforts to control the

spread of sweet potato viruses by controlling the vectors have not been successful (Gibson *et al*., 1997). Both SPFMV and SPCSV and other sweet potato viruses can be controlled using virus free material and controlling weeds, which may serve as alternative hosts of insects and viruses, especially wild *Ipomoea* spp. in and around fields. Isolating new crops a distance from the old mature crops will reduce virus incidence and result in high yielding crops (Gibson and Aritua, 2002).

The use of intercropping to reduce the numbers of infectious vectors attacking the sweet potato crop can help reduce SPVD incidence by delaying SPVD vectors onset and buildup. A sweet potato/maize cropping pattern was found to have a lower SPVD incidence and it can be an option to reduce SPVD damage in the traditional sweet potato farming system. If volunteer sweet potato plants, which may have survived from previous crops, are removed and resistant varieties planted, viral diseases can be minimized (Thompson and Mynhardt, 1986; Karyeija *et al.,* 1998).

In the future, it might be possible to test material in nurseries routinely before using it for propagation. Real-time PCR has been shown to be a more sensitive and specific detection method for these viruses compared to conventional PCR or ELISA assays (Kokkinos and Clark, 2006). For smallholders who cannot benefit from a certified planting material supply system, the capture of seedlings is an efficient way of accessing clean propagules. The fact that sweet potato in Africa is perceived as a crop for the poor, mainly grown by women, has many implications for cultivation of the crop (Kapinga *et al*., 2007). Traditional cultivation practices such as piecemeal harvesting and exchanging planting material freely between neighboring farmers provides for the spread and perpetuation of virus infected material. Farmers obtain planting materials from mature crops which are normally not virus free and on which pesticides are rarely used (Karyeija *et al.,* 1998).

To control spread, it is necessary to make sure that cuttings are collected from healthy plants and to remove diseased plants as soon as they appear. The most convenient means of controlling SPVD is to plant cultivars which have resistance to virus diseases.

Most farmers do not know sweet potato seeds or seedlings and those who do, unfortunately ignore them. The neglect of seedlings by these farmers is likely to hinder the development of more acceptable, SPVD-resistant cultivars and is probably contributing to the establishment of SPVD as a long-term disease problem. In Melanesian countries, on the other hand, farmers traditionally adopt volunteer plants resulting from the spontaneous germination of the numerous seeds produced in

plots where different varieties are associated and it is likely that this approach has a positive impact on the health status of their plants. Other viruses affect sweet potato in East Africa (Kitisha and Gibson, 1999; Thompson and Mynhardt, 1986). The genetic basis of the resistance to these viruses has been investigated in Uganda and Peru. Molecular marker (AFLP and RAPD) studies yielded two genetic markers associated with resistance to SPCSV and SPFMV (Colinet, *et al*., 1994). It is thought that in the presence of both of these viruses, additional genes mediate oligogenic-a phenotypic trait produced by two or more genes working together or multigenic horizontal effects in the sweet potato progenies and breeders are now integrating this information in their schemes combining different sources of resistance (Aritua *et al*., 1998; Kapinga *et al*., 2007).

The development of transgenic sweet potato plants can be another method of controlling sweet potato viruses. The use of cysteine proteinase inhibitor gene (oryza cystatin I) proved to make some sweet potato cultivars tolerant to SPFMV-RC. Although the transgenic lines can still be affected by SPFMV-RC through grafting with SPFMV-RC infected *I. setosa,* it was proven that the multiplication rate of the virus is reduced and the virus cannot be detected directly by either visual observation or by NCM-ELISA (Thottapilly and Rossel, 1988; Cipriani *et al.,* 2000).

Genetic engineering of sweet potato may be possible but its extended application will be limited by resources, multiplication of viruses and their strains, and virus complexes that may alter virus-plant interactions and result in disease development. Meristem-tip culture is a method for eliminating viruses from sweet potato cultivars and is based on the discovery that virus concentrations are lower in plant apices. Together with thermotherapy whereby sweet potatoes are grown at 38-40°C for four to 12 weeks, can possibly give rise to virus free plants (Geleta Dugassa and Tileye Feyissa, 2009; Tekaligh Wondimu *et al*., 2009; Chiu *et al.,* 1982).

Due to its importance, sweet potato germplasm free from known viruses is needed for commercial production, and cultivation practices neglected by many small-scale farmers need to be taken into consideration to prevent further spread. In South Africa, sweet potato varieties are cleaned from viruses through virus elimination and a sweet potato plant improvement scheme, which was initiated in the early seventies. The scheme involves maintenance of disease-free mother stock of the varieties in an insect-free greenhouse and obtaining disease-free plantlets, which are supplied to registered sweet potato vine growers and sweet potato producers (Laurie and Stork, 1997).

7. Objectives

7.1. General Objectives

- To assess the magnitude of sweet potato virus infection and to determine their prevalence in the major producing areas of Ethiopia.

7.2. Specific Objectives

The specific objectives of the survey were to:

- Detect field occurrence of sweet potato viruses.
- Identify the type (s) of existing viruses on the farmers' field.
- Determine the prevalence of excising sweet potato viruses.
- Reveal the existing status of viral distribution in the country.

8. Materials and Methods

8.1. Sweet Potato Sample Collection and Establishment

A total of ten samples showing symptoms of a suspected viral infection and symptomless (if any) 2-4 month old sweet potato vein cuttings were randomly collected per each farmers' field along road sides at an average distance of 6 km from Southern (Sampling areas: Awassa Agricultural Research Center (AARC), Hadiya, Sidama, wolayita, Gamogofa and Kembata-Tembaro) and Eastern (Zones: West and East Hararge) part of Ethiopia, the known sweet potato growing areas in the country (Fig.1). Samples were collected between 26th of October to 31th and 27th of November to 1st of December, 2009 sweet potato growing seasons. A total of 970 (235 symptomatic and 753 asymptomatic) sweet potato cuttings from the farmers' fields and 624 imported *in vitro* orange fleshed plantlets were included in this study.

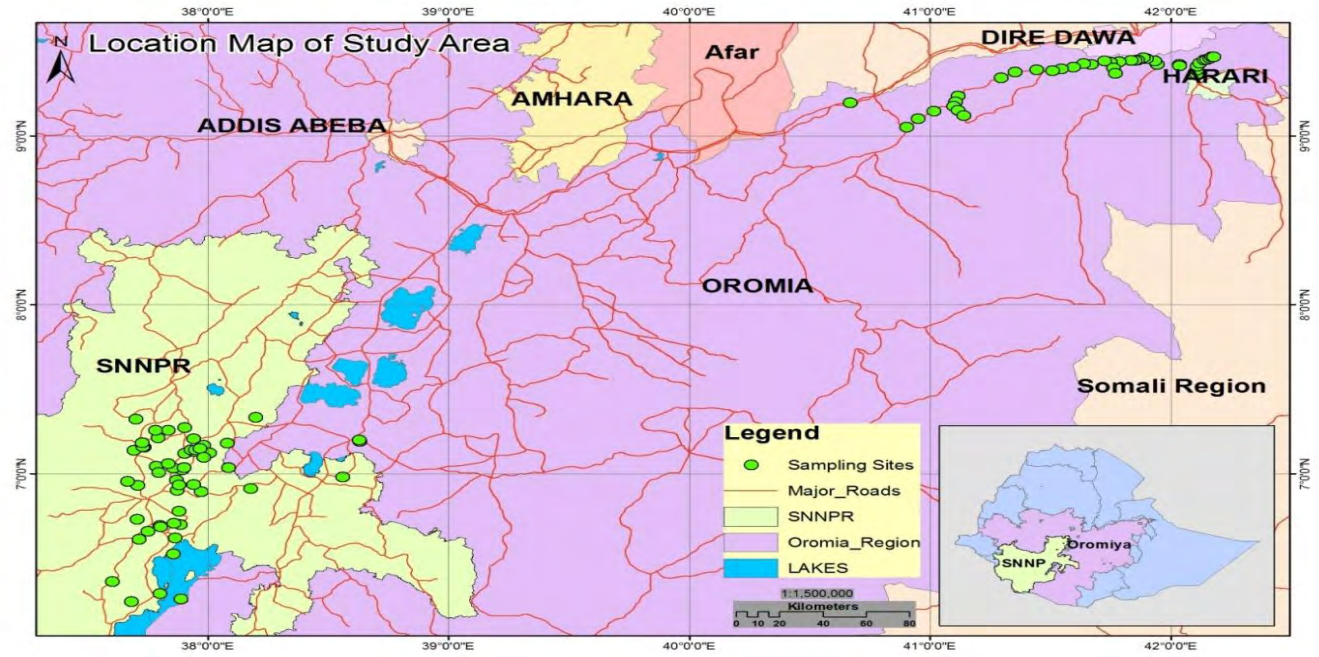

Fig. 1. Map of sweet potato sample collection zones

Symptomatic and asymptomatic vein cuttings of sweet potato plants were kept in separate polyethylene bags labeled with the respective location and brought to Holetta Agricultural Research Center (AHRC) for establishment. Samples were then planted into two subgroups, asymptomatic and symptomatic at a distance of 1m in plastic pots containing sand: soil: cow dung (3:2:1)

(volume: volume: volume) proportion in insect proof screen house and watered every 2 days. Established plants were inspected for any potential vectors every 2 days.

8.2. NCM-ELISA

One centimeter diameter (1cm) leaf disks approximately 0.5 g fresh weight were taken from fully expanded leaves; from top, middle and lower part of each sweet potato plants maintained in the screen house and fully from expanded leaves of imported *in vitro* plantlets for serological assay. Cross-contamination during sample preparation was avoided by inserting leaves in separate plastic bags before taking the leaf disks. Then, the test samples were crushed in 1ml of extraction buffer. The leaf extract was left at room temperature for 15 minutes to let the plant sap phase out (App.1).

For serological detection, the air dried loaded membranes were soaked in 30 ml blocking buffer in separate containers and agitated at a speed of 50-60 rpm for 1hr with the negative and positive control strips. Then, blocking solution was discarded and the membranes were washed with tris-buffer saline (TBS) for 3 minutes. While washing 100 µl of each antibody for SPFMV, SPVG, SPMMV, SPCSV, SPCFV, CMV, SPC-6, SPCaLV, SPLV, and SPMSV was mixed in 30 ml blocking buffer in separate beakers. The TBS solution was discarded and the antibody solution was poured on to each respective membrane and incubated for 4 hrs at room temperature then the antibody solution was discarded and the membranes were washed with tween20 tris-buffer saline (T-TBS) 4 times for 3 minutes each by agitating at a speed of 100 rpm on a shaker. Excess liquid was blotted by sandwiching the membranes between two filter papers. The membranes were placed back to each respective container and was soaked in 30 ml conjugate solution and incubated for 1hr at room temperature. Conjugate solution discarded and washing step was repeated. Finally, 25 ml of substrate solution was poured on to each membrane and incubated at room temperature for 30-90 minute to observe development of purple color (Aritua, *et al*., 1998).

8.3. TAS-ELISA

A hundred and eighty-four samples were tested using triple antibody sandwich enzyme-linked immunesorbent assay (TAS-ELISA) to examine the source of weak reactions observed and to see whether those samples obtained from East and West Hararge showed true reaction for ant- SPFMV during the NCM-ELISA assay. Those samples which showed two times the range value of the

negative control (i.e. negative control was wells that contained only substrate solution) which approximately was from 1.0 nm on the spectrophotometer reading was taken as positive.

Purified Immunoglobulin G (IgG) was diluted in coating buffer at the recommended dilution of 1:1000 proportions 100µl of the mixture was added in to each well and incubated at 37^0C for 3hrs. Plates were washed by soaking for a few minutes with phosphate buffer saline tween20 (PBS-T) 3 times and blotted by placing upside down on tissue paper. 100µl of 2% skim milk in PBS-T was added to each well and incubated at 37% for 30 minutes for blocking. Blocking solution was then discarded; plates were washed and tap dry. Approximately 0.5g fresh weight was taken from fully expanded leaves; from top, middle and lower part of each sweet potato plants.

Then 100µl of aliquots of test samples extracted in extraction buffer was added to duplicate wells and incubate at 4^0C overnight. Washing step was repeated. Monoclonal antibody (mAb) was mixed in conjugate buffer in 1:100 proportions and 100µl was added in to each well and incubated for 3hrs at 37^0C. Washing step was repeated. Rabbit anti-mouse alkaline phosphatase conjugated (RAM-ap) was mixed with conjugate buffer in 1:2500 proportions and 100µl was added to each well and incubated for 2hrs at 37^0C. Washing and blotting, 100µl aliquot of freshly prepared substrate was added and incubation was at room temperature for 30-60 minutes for color development and plates were placed in spectrophotometer adjusted at absorbance of 405 nm (Gibson *et al.,* 1998).

8.4. Grafting

Seed of the indicator plants, *Ipomoea setosa* was planted five weeks before the grafting. Seeds were scarified by soaking in concentrated sulphuric acid (H_2SO_4) for 25 minutes, and then rinsed six times with tap water. Planted in 3:2:1 soil: sand: cow dung (volume: volume: volume) mixture in individual pots. Essential tools used for grafting the sweet potato stems on to the indicator plants include a scalpel with disposable blades, an alcohol lamp for sterilization purposes, and 'Parafilm' to seal the graft. Five weeks old indicator plants were cut back to two true leaves. Then, branches were removed from four symptomless and four symptomatic sweet potato plants obtained from Easter part of Ethiopia. Each containing a node with a fully expanded leaf attached. Then, three nodes were grafted on to a separate indicator plant and the graft was wrapped with 'Parafilm' to prevent desiccation. Two control grafts was made to provide comparisons: by grafting some stems from a known SPFMV infected sweet potato plant. Cross-contamination was avoided during the grafting

process, by dipping the scalpel used for cutting plant material in 75% ethanol and flaming between every graft (Frison, and Ng, 1981).

8.4. Data Analysis

From each respective nitrocellulose membrane purple and from polystyrene microtiter plate yellow color (i.e. 2x the negative control) developed samples were counted as positive. Those samples that did not develop purple or yellow color were considered as negative or healthy. Then, row data was subjected to *SPSS Version 15.0* to estimate frequency of viral incidence and mean of virus and virus-like symptoms in six zones.

Incidence of virus and virus-like symptoms=X and Incidence of virus infection=Y (James, 1974)

$$X = \frac{No\ of\ syptomatic\ Samples\ in\ a\ given\ field}{Total\ \ plants\ in\ the\ field} x100 \text{ and } Y = \frac{No\ of\ infected\ Samples}{Total\ \ plants\ samples} x100$$

9. Result

9.1. Symptomatology

The most commonly observed symptoms were chlorotic spots, mottling, general chlorosis, leaf clearing, leaf distortion, mosaic, purpling, stunting, and thinning and vein chlorosis (Fig. 2). Symptoms on plants that were co-infected with several viruses were typically more severe than on plants infected with a single virus (Fig. 2 and 3). Sweet potato plants that tested positive only for SPCSV had characteristic purple spot symptom (Fig. 2A), and some sweet potato plants exhibited no symptoms. Symptoms associated with only SPFMV infected plants inter-veinal chlorosis and general overall stunting of the plants (Fig. 2B and Fig 3). Plant samples that were only SPVG infected exhibited yellow spotting and mostly no symptoms (Fig. 2C). Plant samples that were sero-positive for both SPFMV + SPCSV showed severe symptoms including leaf distortion, leaf narrowing, stunting of the plant and purpling of older leaves and vein clearing (Fig. 2D). Sweet potato plants that tested positive for SPVG + SPCSV exhibited purple spots and inter-vienal yellow spots and or no observable symptoms (Fig. 2E).(Fig. F, G, H and I) shows vein chlorosis, yellow spotting, and leaf curling virus-like symptoms observed (Fig. J) shows leaf from a healthy plant. Sero-negative sweet potato plants exhibited mild or no symptoms at all (Fig 2J and 3O).

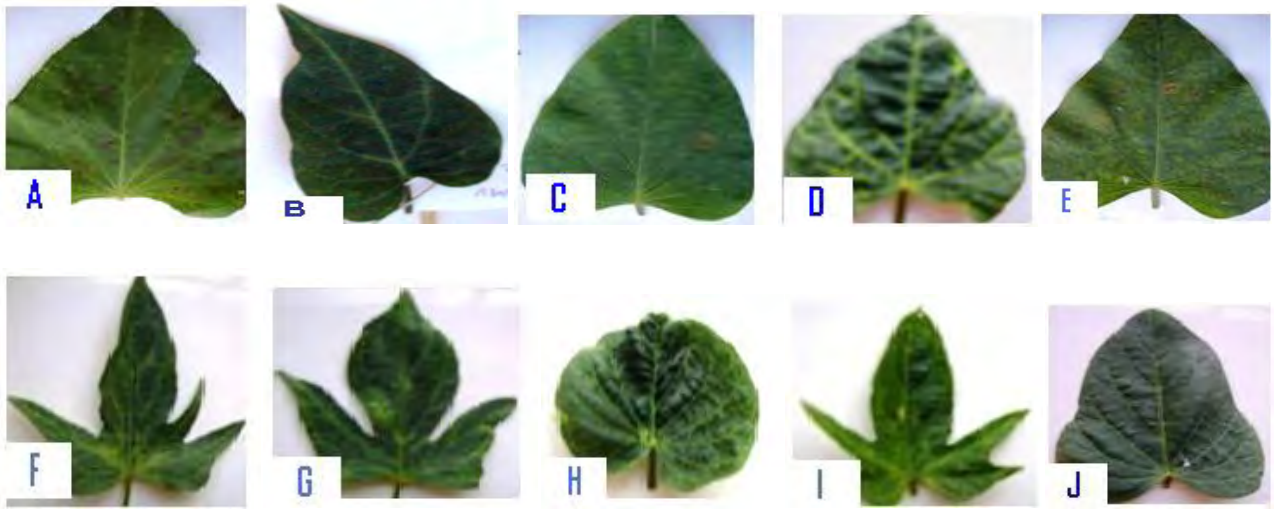

Fig. 2. Virus-like and virus symptoms observed on sweet potato plants collected from Southern and Eastern part of Ethiopia. **A.** Purple spotted leaves of plants infected with *SPCSV*; **B.** Vein chlorosis leaves of plants infected with *SPFMV*; **C.** Chlorotic spotted leaves of plants infected with *SPVG*; **D.** Main vein chlorosis and distorted leaves of plants infected with *SPVD*; **E.** Purple and chlorotic spotted leaves of plants infected with *SPVG+SPCSV* and **F, G, H** and **I.** shows vein chlorosis, yellow spotting, and leaf curling virus-like symptoms observed; **J.** shows leaf from a healthy plant.

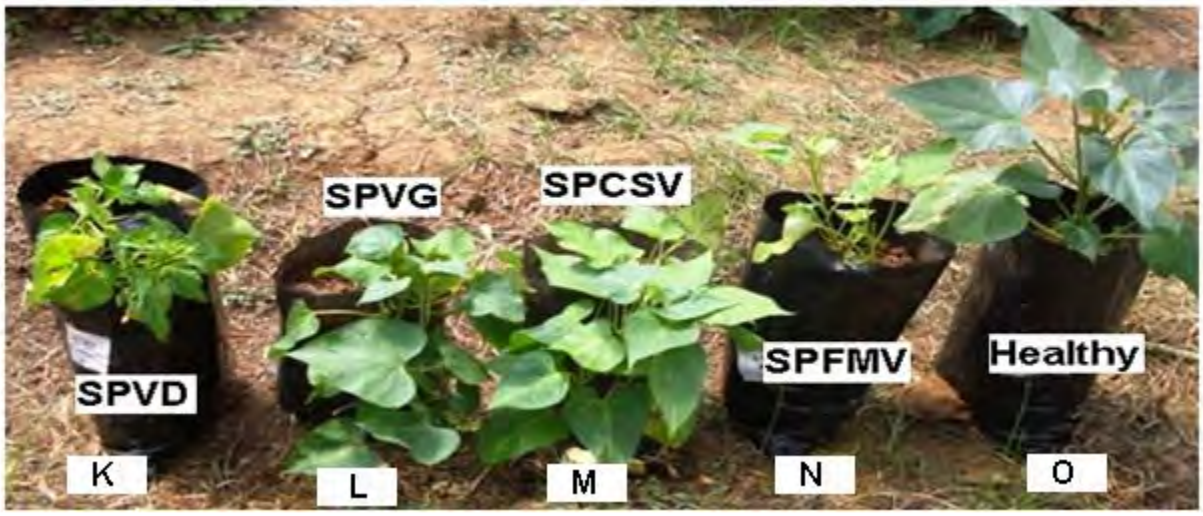

Fig. 3. The degree of vigor between *SPVD, SPVG, SPCSV* and *SPFMV* infected sweet potato plants as compared to the healthy plant, picture taken in the screen hose.

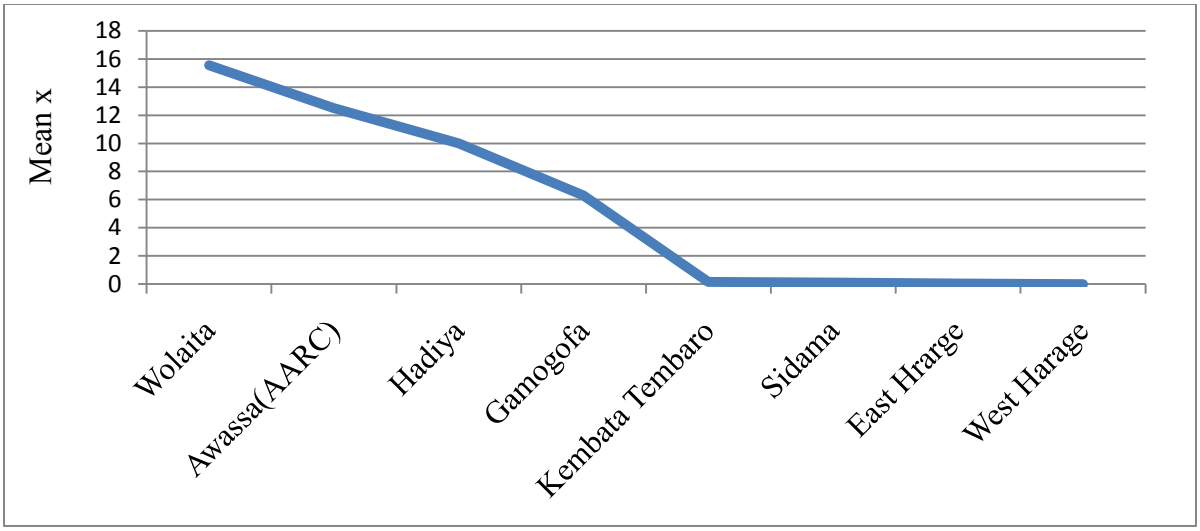

Fig. 4. Mean (x) of field incidences of virus and virus-like symptoms observed during the survey.

The mean (x) of virus and virus-like symptom observed during the field survey was compared and found highest in Wolayita 15.55, followed by Awassa (AARC) 12.5 then in Hadiya 10 and it became highly decreased when it goes from Gamo-Gofa 6.3, Kembata-Tembaro 0.15 Sidama 0.1, East Hararge 0.03 and incidence of symptomatic sweet potato plants drops to zero at Western Hararge (Fig. 4). A total of 8 sweet potato samples obtained from East and West Hrarage were grafted on indicator plants and showed no observable symptoms, whereas the two controls showed veinal chlorosis.

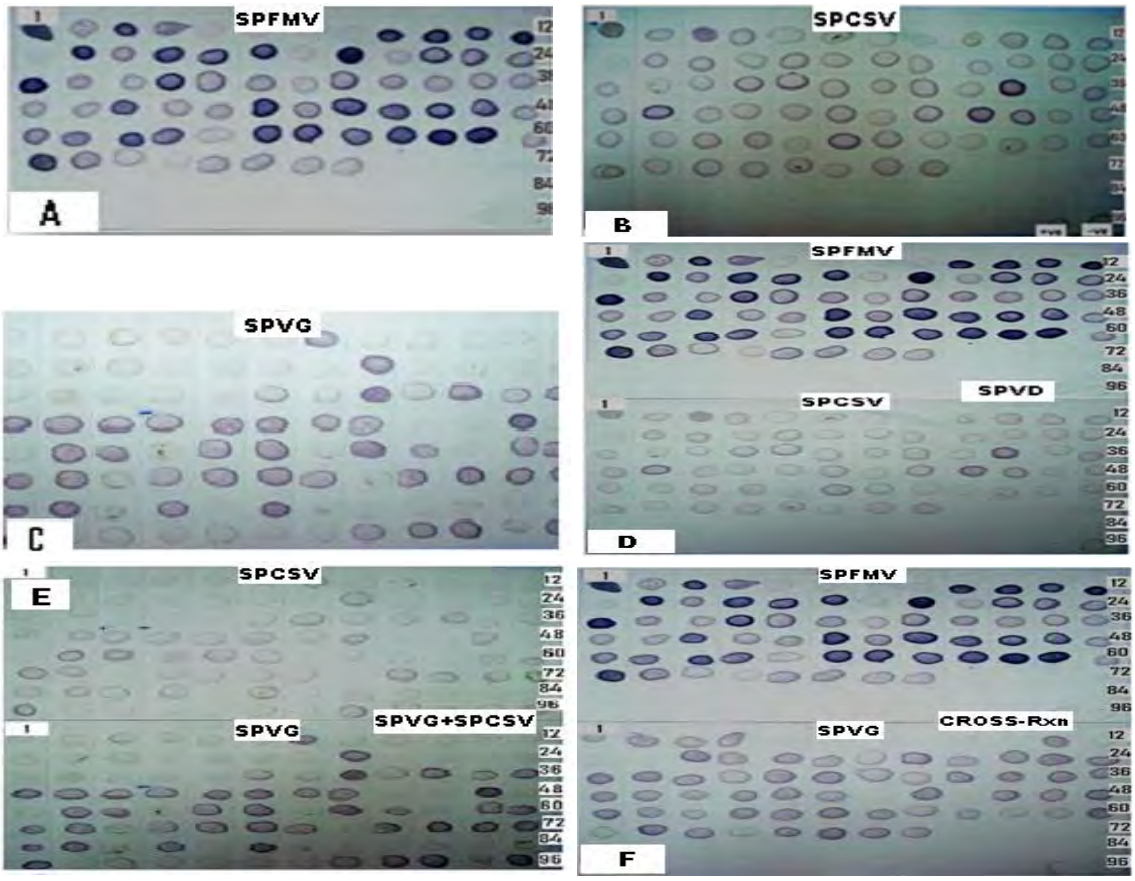

Fig. 5. Purple colors showing samples reacted positive on NCM-ELISA to PAb of: **A.** Anti-*SPFMV*; **B.** Anti-*SPCSV* **C.** Anti-SPVG and **D.** Anti-*SPFMV* and Anti-*SPCSV*=*SPVD* **E.** Anti-*SPCSV*+Anti-*SPVG* **F.** Cross-reaction between Anti-*SPFMV* and Anti-*SPVG* or mixed infection *SPFMV* and *SPVG*.

Fig. 6. TAS-ELISA: Yellow colors showing samples reacted positive to anti-*SPFMV* mAb.

9.2 Incidences of Virus Infection

An average of 20.7% of fields and 19.2% of *in vitro* samples were tested positive for at least one virus and the positive and negative samples were identified by observing the purple color developed

on the NCM-ELISA or yellow color two times the value of absorbance of the negative control on spectrophotometer for TAS-ELISA (Fig. 5; Fig. 6). Of the symptomatic 31.5% sero-negative and 68.5% of sweet potato plant samples reacted positive with antisera of one or more viruses with the frequency of detection being highest in samples obtained from Hadiya and Kebata-Tembaro followed by Awassa (AARC), Wolayita and Gamo-Gofa (Table 1).

From field and *in vitro* asymptomatic samples only 4.1% and 19.2% reacted positive with antisera for at least one virus respectively. Virus diseases were distributed in most of the zones with frequencies of detection ranging from 20-100% and 8.3-30% in the symptomatic and asymptomatic samples respectively. However, most surprisingly none of the symptomatic and asymptomatic sweet potato plant samples from East and West Hararge were found infected with any of the detected viruses (Table 1and 2).

Of 184 sweet potato plant samples tested by TAS-ELISA, 56 samples were those which showed mild reaction to pAb of SPFMV during the NCM assay which also with suspected virus symptoms and, 128 symptomless plants that were obtained from East and West Hararge. It was found that 20 (35.7%) of weakly reacted samples were found positive for anti-SPFMV mAb. Thus, none of the symptomless plants that were obtained from East and West Hararge were sero-positive for mAb of SPFMV on TAS-ELIAS.

Table 1. Proportion of symptomatic and asymptomatic sweet potato plant samples tested positive for at least one virus when assayed serologically by NCM-ELISA from the eight zones of Ethiopia and *In vitro* Plantlets.

	No. Asymptomatic Sweet Potato Plant Samples		**No. Symptomatic Sweet Potato Plant Samples**	
Sampling Areas	**Samples Assayed**	**Percentage (%) Sero-Posetive 1≤ viruses**	**Samples Assayed**	**Percentage (%) Sero-posetive 1≤ viruses**
AWASSA(AARC)	10	30	10	90
SIDAMA	5	0	5	20
HADIYA	5	0	5	100
WOLAYITA	206	9.2	134	83.6
GAMO GOFFA	96	8.3	34	67.6
KEMBATATEMBARO	9	0	11	100
EAST HARAGE	314	0	36	0
WEST HARARGE	90	0	0	0
**Melkassa	624	19.2	0	0
Total	**1359**		**235**	

**Imported

Three sweet potato infecting viruses tested were detected by NCM-ELISA namely SPFMV, SPCSV and SPVG. They were detected in both symptomatic and asymptomatic sweet potato field samples and SPFMV and SPCSV were detected in the *in vitro* samples. However, none of the other viruses, SPMMV, SPCFV, SPLV, SPC-6, SPCaLV, SPMSV and CMV were detected (Table 2). The frequency of detection was higher in symptomatic plants than asymptomatic in the case of field plant samples and yet, only for the symptomless *in vitro* samples.

From the total symptomatic and asymptomatic samples 133 (56.6%) and 13 (1.8%) respectively were reacted for Anti-SPFMV revealing the fact that SPFMV is the most prevalent virus detected. SPCSV is the second most frequent virus being detected in 11 (48.9%) of symptomatic and 10 (1.4%) asymptomatic samples respectively. SPVG being the third yet, low frequent virus being detected in 28 (11.9%) symptomatic and 16 (2.1%) asymptomatic plant samples. SPVD was the most prevalent co-infection and was detected in 88 (37.4%) symptomatic plant samples and 2 (0.27%) asymptomatic plant samples. The second prevalent dual infection detected was SPCSV+ SPVG in 7 (0.9%) asymptomatic and 25 (10.2%) symptomatic samples (Fig. 7).

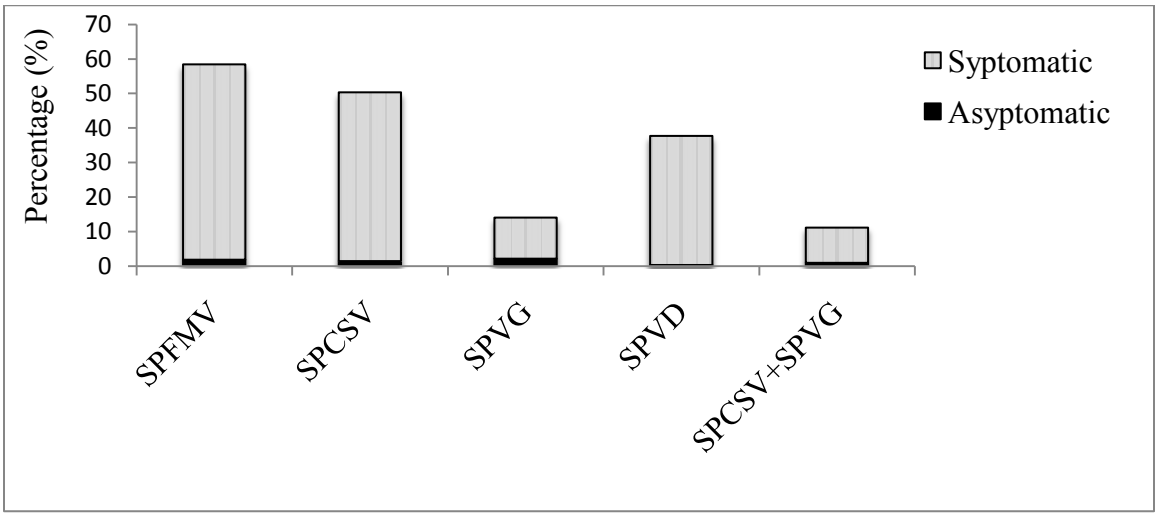

Fig. 7. Prevalence of single and dual infection detected during the NCM-ELISA assay in samples obtained from 97 fields.

Finally, from the total of *in vitro* plantlets 120 (19.2%) were reacted for Anti-SPFMV and only 1 (0.2%) reacted for Anti-SPCSV. One undeniable fact that has occurred during the NCM-ELISA assay was 90 (61.6%) of the symptomatic sweet potato plant samples showed cross-reaction between Anti-SPFMV and Anti-SPVG pAb (Fig. 5). The frequency of detection of virus infections varies at each sampled locations (Table 1). Of the eight sampled locations the frequency of

detection of SPFMV was very high at the five locations namely Hdiya 5 (100%) followed by Kembat-Tembaro 11 (100%), Awassa (AARC) 7 (70%), Gamo-Gofa 23 (67.6%) and Woliyta 86 (64.7%) of the symptomatic plant samples and from the asymptomatic plant samples Gamo-Gofa being the highest 6 (6.3%) followed by Wolaiyta 7 (3.4 %.)

Of the symptomatic plant samples SPCSV detected was lower than SPFMV, with the frequency 5 (100%), 10 (90.9%), 91 (67.9%), 8 (23.5%) 1 (10%) Hadiya, Kebata-Tebaro,Wolayita, Gamo-Gofa and Awassa (AARC) respectively. SPCSV was detected in fewer asymptomatic plant samples obtained from Woliyta 7 (3.4%) and Gamo Gofa 3 (3.1%). The frequency of detection of SPVG was much restricted than the other two viruses and found at only three locations Awassa (AARC) 2 (20%) and 3 (30%), Wolaiyta 24 (17.9%) and 11 (5.3%) and Gamo-Gofa 3 (5.9%) and 2(2.1%) from symptomatic and asymptomatic plant samples respectively (Table 2). SPVG was relatively the most frequent virus detected in asymptomatic samples. Furthermore, the occurrence of the three viruses was also higher in Wolayita and Gamo-Gofa than in the other zones (Table 2).

Table 2. Proportion of symptomatic (S) and Asymptomatic (A) sweet potato plant samples from the eight zones of Ethiopia and *In vitro* Plantlets reacted positive for different viruses.

	No. of Plants Assayed		**Percentage (%) *SPFMV***		**Percentage (%) *SPCSV***		**Percentage (%) *SPVG%***	
Sampling Areas	**S.**	**A.**	**S**	**A**	**S**	**A**	**S**	**A**
AWASSA(AARC)	10	10	70	0	10	0	20	30
SIDAMA	5	5	20	0	0	0	0	0
HADIYA	5	5	100	0	100	0	0	0
WOLAYITA	134	206	64.2	3.4	67.9	3.4	17.9	5.3
GAMO-GOFA	34	96	67.6	6.3	23.5	3.1	5.9	2.1
KEMBATATEMBARO	11	9	100	0	90.9	0	0	0
EAST HARAGE	36	314	0	0	0	0	0	0
WEST HARARGE	90	0	0	0	0	0	0	0
**MELKASSA	0	624	0	19.2	0	0.2	0	0
Total	**235**	**1359**						

**Imported

9.4. Single and Mixed Infections

Incidence of mixed and single infection also varied accordingly (Fig 8 and 9), in each zones. From asymptomatic plant samples Gamo-Gofa 5 (5.2%) was the highest followed by Awassa (AARC) 6(2.9%) and from the symptomatic plant samples Gamo-Gofa 23 (67.6%) Awassa 6 (60%) Sidama

1(20%) Wolaiyta 17 (12.7%) and Kebata-Tembaro 1 (9.1%) were reacted positive for Anti-SPFMV. The frequency of Single SPVG latent infection was higher at Awassa (AARC) 3 (30%) then followed by Wolaiyta 3 (1.5%).

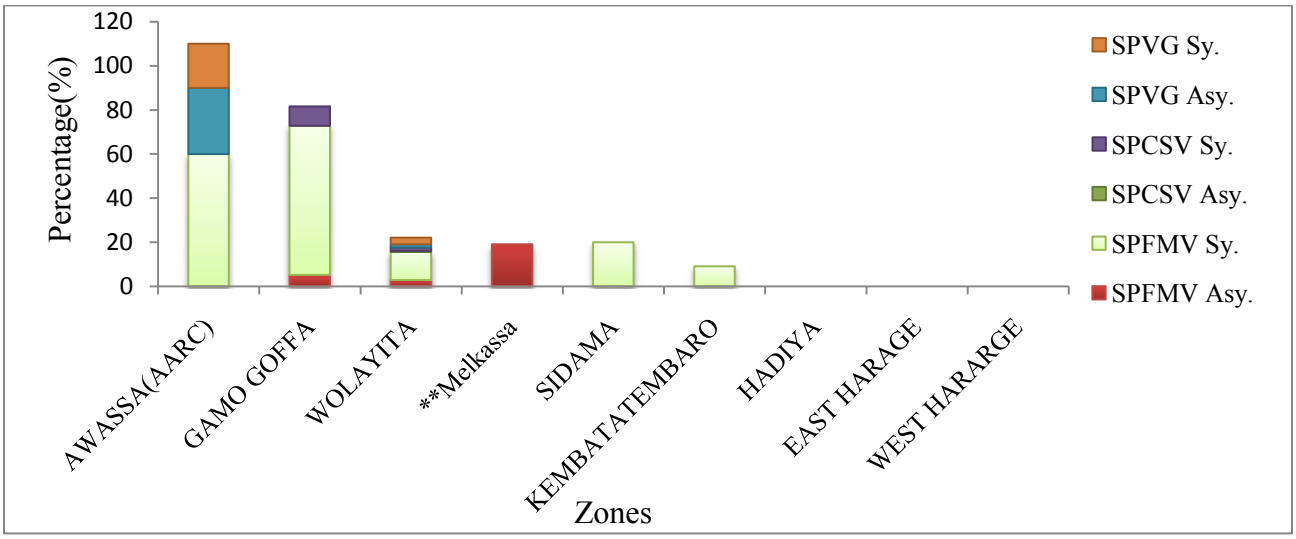

Fig. 8. Proportion of single virus infection detected by NCM-ELISA assay from the eight zones of Ethiopia and *in vitro* plantlets.

Symptomatic plant samples which were sero-posetive for only Anti-SPVG were 2 (20%) and 4 (3%) in the former zones respectively. Anti-SPCSV positive asymptomatic and symptomatic plant sample was not detected in most zones in single infection with the exception of Wolaiyta 1 (0.5%), and 2 (1.5%) asymptomatic and symptomatic respectively and Gamo-Gofa 3 (8.8%) symptomatic plant samples while, frequency of detection was still very low. Therefore one can infer the rates of detection of viruses were higher in the symptomatic plant samples than the asymptomatic except in the case of SPVG in samples obtained from Awassa (AARC).

Furthermore, co-infection infections observed were SPFMV+SPCSV=SPVD and SPCSV+SPVG (Fig.9). Incidences of SPVD infection in the symptomatic plant samples were high in Hadiya 5 (100%) then followed by Wolaiyta 69 (51.1%), Kembata-Tembaro 10 (50%), Awassa (AARC) 1 (10%) and Gamo-Gofa 3 (8.8%). Latent SPVD observed was only in sweet potato samples obtained from Wolayita, Gamo-Gofa and Melkassa 1 (0.5%), 1 (1.04%) and 1 (0.2%) respectively.

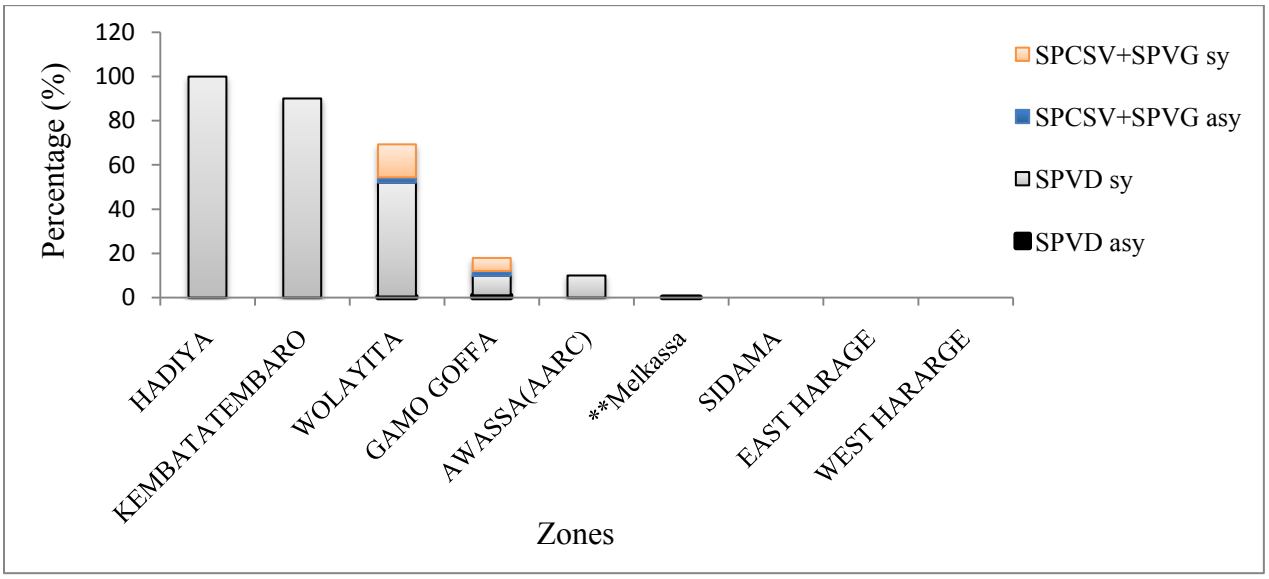

Fig. 9. Proportion of mixed virus infection detected by NCM-ELISA assay from the eight zones of Ethiopia and *in vitro* plantlets.

The second type of mixed infection observed was SPVG+SPCSV and the frequency in Wolaiyta and Gamo-Gofa was 5 (2.4%) and 2 (2.2%) from the asymptomatic plant samples and 20 (15%) and 2 (5.9%) of the symptomatic plants respectively. Frequency of both single and mixed infections was high in Wolaiyta and Gamo-Gofa. Incidence of SPCSV+SPVG infection from the asymptomatic plant samples was higher when compared to SPVD. Finally, incidence of SPVD was higher in Hadiya, Kembata-Tembaro and Woliyta and very low in Awassa (AARC).

10. Discussion

The survey was done in order to asses current viral distributions and to identify the types of viruses in the farmers' fields using serological techniques: grafting, triple antibody sandwich enzyme-linked immunosorbent assays (TAS-ELISA) was done for SPFMV, and nitrocellulose membrane enzyme-linked immunosorbent assays (NCM-ELISA) using kits supplied by the International Potato Centre (CIP), Lima, Peru. Kits from CIP contain antisera to detect up to 10 of the over 20 viruses that have been reported infecting sweet potato in different parts of the world. Therefore, the survey did not include viruses for which antiserum is not available and was similar to viral assessment studies made in most of East African region (Mukasa *et al*., 2003). As in the current study it was reported that the most commonly observed symptoms were chlorotic spots, mottling, general chlorosis, leaf clearing, leaf distortion, mosaic, purpling, stunting, thinning and vein chlorosis (Tameru Alemu, 2004; Nyaboga *et al*., 2008; Ndunguru *et al*., 2009).

Viruses have previously never been major limiting factors in sweet potato production in Ethiopia. The first report of a virus on sweet potato in the country was made over two decades ago by electron microscopy of sweet potato plants with mosaic symptoms from Nazreth, the virus being tentatively identified as SPFMV (SPL, 1986). Recently, however, Tameru Alemu, (2004) reported a high incidence of SPFMV in some fields and the occurrence of another virus named as SPVG mainly from Wolayta zone and SPV2 in research stations in Ethiopia (Adane Abrahme *et al.,* 2007). The current study also reported the same types of viruses having relative prevalence with the previous studies except for SPV2.

Surveys done in Ethiopia, Uganda, Kenya, Zimbabwe and Tanzania found sweet potato plants with symptoms resembling those caused by viruses, but which did not react with any of the antisera used. Such plants could be infected with viruses that have been described but were not tested for in the studies and/or viruses that have not yet been described in sweet potato and another possible suggestion reported was the presence of phenolic compounds, latex in the plant tissue and inhibitors that adversely affect the serological detection and symptoms caused by non-viral factors (Mukasa *et al*., 2003; Tameru Alemu, 2004; Ndunguru and Kapinga, 2007; Ndunguru *et al*., 2009). This phenomenon is also confirmed in the present study. The development of purple or yellow color was the indication of infected samples during the assay as indicated by other reports (Gutierrez *et al*., 2003; Converse and Martin, 1990).

Three viruses namely SPFMV, SPVG and, SPCSV for the first time, were detected in sweet potato plants collected from farmers' fields in main growing areas of Ethiopia. The same types of viruses that were identified in this study, (i.e. SPFMV, SPCSV and SPVG), have also been detected in other surveys (Colinet *et al*., 1996; Tameru Alemu, 2004; Mukasa *et al*., 2003; Ndunguru and Kapinga, 2007; Ndunguru *et al*., 2009). The most frequent virus detected was SPFMV which is equivalent to earlier survey confirming that SPFMV is widespread everywhere sweet potato is grown (Gibson *et al*., 1998; Mukasa *et al*., 2003; Kokkinos and Clark, 2006; Ndunguru and Kapinga, 2007). Thus, in this survey the spread is so restricted to the Southern part of the country. Gibson *et al*. (1998) reported that SPFMV on its own causes mild or no symptoms in East African sweet potato cultivars. The study this observation was confirmed.

Sweet potato chlorotic stunt virus SPCSV was the second most prevalent virus and was detected in both symptomatic and asymptomatic plant samples. On its own it exhibits obvious or mild symptoms but in mixed infections, shows very severe symptoms. This is equivalent to previous reports from East Africa regions: USA, Tanzania, Ethiopia, Kenya and Uganda (Gibson *et al*., 1998 Mukasa *et al*., 2003; Tameru Alemu, 2004; Ndunguru *et al*., 2009; Kokkinos and Clark, 2006; Ndunguru and Kapinga, 2007; Nyaboga *et al*., 2008). In this study SPCSV occurred most frequently in mixed infections with SPFMV than alone that is also comparable with the other reports (Mukasa *et al*., 2003; Ndunguru and Kapinga, 2007; Nyaboga *et al*., 2008).

SPVG has a narrow distribution and was rarely encountered, which is in agreement with previous reports from Egypt, and South Africa and other countries in East Africa such as Ethiopia and Kenya (Ishak *et al*., 2003; Tameru Alemu, 2004; Kokkinos and Clark, 2006).

A large number of sweet potato plant samples infected with SPFMV were also reacted positive to anti-SPVG during the NCM-ELISA assay. The cross-reaction between antisera of the two viruses was may be due to the ability to elicit comparable antibody in the immune system of the mammal in which they were introduced as an antigen. Which other reports also confirmed this fact stating that, the coat protein of SPVG is longer than that of SPFMV, but it is closely related to SPFMV and thus, it is considered as a potyvirus. However, there is also a possibility that the two viruses may have co-infected sweet potato plants (Salazar and Fuentes, 2000; Ishak *et al*., 2003).

There was a very weak reaction observed during the NCM-ELISA assay even if it was rechecked with a more sensitive TAS-ELISA used in the current study. As suggested in other equivalent

reports this might be due to, serological assays are known to be somewhat insensitive in detecting some viruses, especially when done directly from sweet potato as the plant contains unusually large but variable concentrations of a number of substances such as latex, polyphenols, and polysaccharides in the plant tissue and inhibitors that adversely that interfere with the assays and symptoms caused by non-viral factors as it has been reported or may be due to undetectable viral titer (Gibson *et al*., 1998; Salazar and Fuentes, 2000). Grafting was also one of the tools employed in the current study for samples brought from Eastern part of the country. Thus, the present study did not confirm the presence of any infection even if virus-like symptoms existed. That however, may be due to symptoms are sporadic and brief on the indicator plants such as *Ipomoea setosa* as suggested by Frison, and Ng, (1981).

The widespread occurrence of SPFMV as compared to the other two viruses might be related to the way farmers select their planting materials and/or via aphid *(Myzus persicae* Sulz.) transmission (Ames *et al.,* 1997). Since sweet potato plants that are singly infected with SPFMV, SPCSV and SPVG exhibit mild or no symptoms. Therefore, farmers may not be able to distinguish and exclude such infected cuttings from the planting materials they select for the next crop. Thus, maintaining and favoring persistent spread of these viruses (Njeruet *et al*., 2008; Nyaboga *et al*., 2008). As managing viruses infecting sweet potato will require knowledge on etiology and ecology of the viruses. Information on the control of viruses and how viruses infect plants is lacking among resource-poor farmers (Di Feo *et al.*, 2000). The present study also elucidated the above fact. This phenomenon forms the root of suggestion that SPFMV, SPCSV and SPVG are the most important viruses that infects sweet potato in Ethiopia.

Mixed virus infections in sweet potato are a common phenomenon (Gibson *et al*., 1998). The commonness of co-infection is also confirmed in the present study. Sweet potato virus disease (SPVD), the disease caused by concurrent infection of SPFMV and SPCSV is severe as in the other East African countries. The present result also agree with findings from previous surveys in Kenya (Nyaboga *et al*., 2008), Uganda (Mukasa *et al*., 2003), Tanzania (Ndunguru and Kapinga, 2007; Ndunguru *et al*., 2009) and Rwanda (Njeruet *et al*., 2008). The common occurrence of SPVD in Ethiopia could be related to the practice of farmers using vines from their existing gardens as planting materials, and without sanitary control as a result facilitating spread of the disease.

The other dual viral infection observed was SPCSV+ SPVG yet less frequent as was reported in a previous study in Uganda (Ndunguru and Kapinga 2007; Ndunguru *et al*., 2009), although it was

not reflected as a commonly found mixed infection in sweet potato plants. The co-occurrence of these viruses may be due to mixed transmission of the two viruses by their common whitefly (*Bemisia tabaci*) (Clark and Moyer, 1988; Ateka *et al*., 2005; Valverde *et al*., 2004). The two viruses were mostly detected in symptomatic plants but it is not known whether synergism exists in co-infected plants (Valverde *et al*., 2004; Kokkinos and Clark, 2006). The results of this study also strengthen previous findings of severe symptoms being associated with co-infections with multiple viruses (Gibson *et al*., 1998a; Di Feo *et al*., 2000; Mukasa *et al*., 2003). However, it should be clear that not all severe symptoms on sweet potato are due to synergistic effect of mixed infections (Salazar and Fuentes, 2000).

11. Conclusion

Incidences of Virus and virus-like syptomes were high in Wolayita, Awassa (AARC), Hadiya and low Gamo-Gofa, Kembata-Tembaro, Sidama, East Hararge and it drops to zero in Western Hararge. Sixty eight point five percent (161) of the 235 symptomatic plants tested were positive with at least one of the virus specific antisera used, which suggest that the three viruses detected are principally responsible for the viral diseases of sweet potato in Ethiopia.

A number of symptomatic plants 74 (31.5%) sero-negative although the symptoms resembled those caused by viruses. It is possible that more viruses or virus-like agents than the ten viruses tested in this study infect sweet potato in Ethiopia, another possibility is also that the detection is not successful although the viruses exist. Some symptomless plants from field and *in vitro* 4.1% and 19.2% reacted with antisera for at least one virus respectively. This might be due to the ability of the plants to tolerate the effects of virus infection.

From the total symptomatic and asymptomatic samples 56.6% and 1.8% respectively were reacted positive for Anti-SPFMV revealing the fact that SPFMV the most frequently detected virus. SPCSV was the second most frequent virus being detected in 48.9% of symptomatic and 1.4% asymptomatic samples respectively. SPVG being the third yet, low frequent virus being detected in 11.9% symptomatic and 2.1% asymptomatic plant samples. From imported *in vitro* plantlets a total of 19.2% were reacted positive for Anti-SPFMV and only 0.2% reacted for Anti-SPCSV. SPFMV was detected in most of the zones surveyed, whereas SPCSV in five and SPVG in three zones that were surveyed.

Sweet potato virus disease SPVD was the most common co-infection observed in large symptomatic plant samples and fewer asymptomatic plant samples. The second dual infection was SPCSV + SPVG and found in bigger symptomatic samples than asymptomatic samples. Thus, one can conclude that the rate of detection of viruses were higher in the symptomatic sweet potato plants than asymptomatic except in SPVG infected samples obtained from Awassa (AARC). Detection of SPCSV+SPVG infection from the asymptomatic plant samples was higher when compared to SPVD.

In addition, SPFMV and SPCSV was the most common combination of mixed infection and detected in only two symptomless plants, proving that symptoms are obviously severe in SPVD infected sweet potato plants. Sweet potato virus disease (SPVD) was high in Hadiya, Kebbata-

Tembaro and Wolaiyta zones. However, incidence of the deferent viruses was high in Wolaiyta and Gamo-Gofa. None of symptomatic and symptomless sweet potato plant samples collected from Eastern Hararge and Western Hararge was any virus infection found.

This study has provided a quantitative assessment of single and co-occurrence of viruses in sweet potato plants in Ethiopia, taking into account the major growing areas. Some single infections caused by SPFMV, SPVG or SPCSV, and also some mixed infections observed exhibit mild or no symptoms this could be a potential risk for the widespread of sweet potato viral diseases in the near future especially in those zones where there is high SPVD incidence unless some preventive measures taken.

12. Recommendation

The outcome of the present study reveals the possibility that other, as yet unknown, or less characterized viruses not detected in this study might occur in the surveyed areas. Since some of plants established in the screen house showed virus-like symptoms but were sero-negative with the antibodies used. Thus, further studies are required to identify the cause of virus-like symptoms in sweet potato plants. SPCSV was associated with reasonably severe symptoms in mixed infections with SPFMV and SPVG. SPFMV or SPVG or SPCSV alone and some dually infected sweet potato plants exhibited mild or no symptoms. This could be a potential risk for the widespread of sweet potato viral diseases in the near future. For this reason, awareness should be created around sweet potato growers regarding symptom identification, especially on how to select sources of propagules for the next cropping and on how to manage widespread of viral diseases.

Internal quarantine measures should be initiated to avoid the movement of sweet potato virus infected materials from those areas where the sweet potato virus disease, to areas like Eastern and Western Hararge where no viral infections were detected. This calls for the attention of the concerned bodies to control the transportation of tubers and/or any vein sweet potato cuttings from the Southern part of the country to Hararge. This finding highlights the importance of targeting resistance to SPVD and the other viruses in either conventional or nonconventional breeding programs as a means of virus disease management. Plant tissue culture through meristum culture in combination of thermo/chemotherapy should be employed in a large scale to produce virus free materials and using seeds as propgules than vein cuttings in order to minimize persistence and spread of viral diseases.

Highly sensitive virus detection methods as grafting and/or molecular techniques should be used extensively to detect a very low viral titer and to avoid the confusion due to: mild reactions observed during ELISA assay and to find out the causes of virus-like symptoms. Creating awareness among the Ethiopian farmers regarding the etiology, ecology of the viruses, how viruses infect plants and on the control measures that should be taken. Imported *in vitro* plantlets for research purpose should be tested for virus infections before mass-propagation and release to farmers because they mostly do not show associated symptoms of viral infections. Finally, host range and associated vector incidences with each virus should be investigated specially in high SPVD incident areas.

13. References

Abad, J.A. and Moyer, J.W.(1991). Detection and Distribution of Sweet Potato Feathery Mottle Virus (SPFMV) in Sweet Potato by *in vitro* Transcribed RNA Probes (Rhiboprobes), Membrane Immunobinding assay (MIBA), and Direct Blotting. *Phytopathol.* **82**:300-305.

Adane Abraham, Alemu Lencho and Berhanu Bekele (2007). Viruses Threatening Sweet Potato and Soybean Improvement and Production in Ethiopia ***In****: Abstract of Proceedings Presented at the 15th Annual Conference of the Plant Protection Society of Ethiopia*. pp. 1-2, November, 2007, EIAR.

Alicai, T., Fenby, N. S., Gibson, R. W., Adipala, E., Vetten, H. J., Foster, G. D. and Seal, S. E. (1999). Occurrence of Two Serotype of Sweet Potato Chlorotic Stunt Virus in East Africa and their Associated Differences in Coat Protein and HSP70 Homologue Gene Sequences. *Plant Pathol.* **48:**718-726.

Ames, T., Smit, N. E. J. M., Braun, A. R., O'Sullivan, J.N. and Skoglund, L.G. (1997). Sweet Potato: Major pests, diseases, and nutritional disorders. International Potato Center (CIP). Lima, Peru.

Aritua, V., Adipala, E., Carey, E. E. and Gibson, R. W. (1998). The Incidence of Sweet Potato Virus Disease and Virus Resistance of Sweet Potato Grown in Uganda. *Ann. Apple. Biol.* **132:**399-411.

Aritua,V., Alicai T., Adipala E., Carey E. E. and Gibson W. R. (1998). Aspects of Resistance to Sweet Potato Virus Disease in Sweet Potato. *Ann. Appl. Biol.* **132:**387-398.

Assefa Tofu, Teshome Anshebo, Engida Tsegaye and Tesfaye Tadesse, (2007). Summary of Progress on Orange-Fleshed Sweet Potato Research and Development in Ethiopia. *Proceedings of the 13th ISTRC Symposium*. pp. 728–731.

Ateka, M. E, Njeru, W. R, Kibaru, G. A Kimenju, J. W, Barg, E., Gibson, W .R and Vetten, J. H. (2005). Identification and Distribution of Viruses Infecting Sweet Potato in Kenya. *Ann. of Appl. Biol.* **144 (3)**371 – 379.

Atkey, P.T. and Brunt, A.A. (1987). Electron Microscopy of an Isometric Caulimo-Like Virus from Sweet Potato *(Ipomoea batatas). Phytopathol.* **118:**370-376.

Austin, D. F. (1988). The Taxonomy, Evolution and Genetics Diversity of Sweet Potatoes and Reared Wild Species. In Exploration, Maintains, and Utilization of Sweet Potato Genetic Resources. (Gregory, P. ed.) International Potato Center, Lima, Peru, pp. 27-60.

Census Bureau, International Data Base. (2009). Countries and Areas Ranked by Population (**http://www.census.gov/cgi-bin/ipc/idbrank.pl**), (Accessed: on 11 June, 2010).

Chavi, F., Robertson, A.L. and Verduin, B.J.M. (1997). Survey and Characterization of Viruses in Sweet Potato from Zimbabwe. *Plant Dis.* **81:**1115-1122.

Chiu, R. J. Liao, C. R. and Chung, M.L. (1982). Sweet Potato Virus Diseases and Meristem Culture as Means of Disease Control in Taiwan. ***In:*** *Sweet potato*: *Proceedings of the First International Symposium.* pp.169-176. (Shanhua, Tainan, Taiwan, China. Villareal, R.L. and Griggs, T.D. eds.). Asian Vegetable Research and Development Center.

Cipriani, G., Fuentes, S., Bello, V., Salazar, L. F., Ghislain, M. and Zhang, D.P. (2000). Expression of Rice Cysteine Proteinase Inhibitors Gene to Develop Resistance Against Sweet Potato Feathery Mottle Virus. ***In:*** *International Workshop on Sweet Potato Cultivar Decline Study*. pp. 14. (Nakazawa, Y. and Ishiguro, K. eds.) Dept. of Upland Farming, Kyushu National Agricultural Experiment Station, Japan.

Clark, M. F. and Bar-Joseph, M. (1984). Enzyme Immunosorbent Assay in Plant Virology. ***In:*** *Methods in Virology*, Vol. 7. (Maramaroch, K & Koprowski, H. eds.). Academic Press Inc., London.

Clark, A. C. and Moyer, W. J. (1989). Compendium of Sweet Potato Diseases. *J. Agri. Sci.* **112:**433-434.

Cohen, J. and Loebestein, G. (1991). Role of Whitefly-Transmitted Agent in Infection of Sweet Potato by Cucumber Mosaic Virus. *Plant Dis.* **75:**291-292.

Cohen, L., Milgram, M., Antignus, Y., Pearlsman, M., Lachman, O. and Loebestein, G. (1997). *Ipomoea* Crinkle Leaf Curl caused by a Whitefly-Transmitted Gemini-Like Virus. *Ann. Apple. Biol.* **131:**273-282.

Colinet, D., Kummert, J. and Lepoivre, P. (1994). The Complete Nucleotide Sequences of the Coat Protein Cistron and the 3 'non-coding Region of a Newly-Identified *Potyvirus* Infecting Sweet Potato, as Compared to those of Sweet Potato Feathery Mottle Virus. *Arch. Virol.* **139:** 327-336.

Colinet,D., Nguyen, M., Kummert, J. and Lepoivre, P. (1998). Differentiation Among Potyviruses Infecting Sweet Potato Based on Genus and Virus-Specific Reverse Transcription Polymerase Chain Reaction. *Plant Dis.* **82:**223-229.

Colinet, J., Kummert, P. and Semal, L. (1994). Identification of Distinct Potyviruses Mixedly Infected Sweet Potato by the Polymerase Chain Reaction with Degenerate Primers. *Phytopathol.* **84:**65-69.

Converse, R. H. and Martin, R. R. (1990). ELISA Methods for Plant Viruses. ***In:*** *Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens*: *A Laboratory Manual*. pp. 179-196. (Ball, E. R. and De Boer, S. eds.). APS Press, Minnesota. USA. Hampton.

Di Feo, L., Nome, S. F., Biderbost, E., Fuentes, S. and Salazar, L. F. (2000). Etiology of Sweet Potato Chlorotic Dwarf Disease in Argentina. *Plant Dis.* **84:**35-39.

Dube C. H. (2004). *A Text book of Fungi, Bacteria and Viruses.* 2nd ed. Vikas Publishing House Pvt. Ltd. India.

Ewell, P. T. and Mutuura, J. (1991). Sweet Potato in the Food System of Eastern and Southern Africa. ***In:*** *Tropical Root Crops in a Developing Economy*. *Proceedings of the 9th Symposium of the International Society for Tropical Root Crops.* Accra.

Fauquet, M. C. and Mayo, M. A. (1999). Abbreviations for Plant Viruses Names. *Arch.virol.* **144:**1267-1268.

Frison, A. E. and Ng, Y. S. (1981). Elimination of Sweet Potato Virus Disease Agents by Meristem Tip Culture. *Inter. J. Pest Manag.* **27:** 452 – 454.

Fuenetes S. G. and Salazar F. L. (2003). Sweet Potato Disease SPVD: Distribution, Incidence and Effect on Sweet potato yield in Peru. *Plant Dis.* **87:**297-302.

Fuglie, K. O. (2007). Priorities for Sweet Potato Research in Developing Countries: Results of a Survey. *HortSci.* **42:**1200–1206.

Geleta Dugassa and Tileye Feyissa (2009). Meristume Culture and Thermotherapy of Selected Sweet Potato (*Ipomoea batatas* (L.) Lam.) to Produce Virus Free Planting Materials. MSc. Thesis, Addis Ababa University, Faculty of Science.

George, N. A. (2005). P*lant Pathology, 5th ed.* Elsevier Academic Press, London, pp. 724-822.

Gibson, R. W. and Aritua, V. (2002). The Perspective of Sweet Potato Chlorotic Stunt Virus in Sweet Potato Production in Africa: A review. *Afri. Crop Sci.* **10:**281-310.

Gibson, R.W. Kaititsha, G.C. and Randrianairoarivony J. M. (1998). Identification of the East African Station of Sweet Potato Choleric Stun Virus as a Major Component of Sweet Potato Virus Disease in South Africa. *Plant Dis*. **132:**10-63.

Gibson, R.W. Mpembe, I., Alicai, T. Carey, E.E., Mwanga, R. O. M. Seal, S. K. and Vetten, H. F. (1998). Symptoms, Aetiology and Serological Analysis of Sweet Potato Virus Disease in Uganda. *Plant Pathol*. **47:**95-102.

Gibson, R.W., Mwanga, R. O. M., Kasule, S., Mpembe, I. and Carey, E. E. (1997). Apparent Absence of Viruses in the Most Symptomless Field-Grown Sweet Potato in Uganda. *Ann. Apple. Biol.***130:**481-490.

Gutierrez, D. L., Fuentes, S. and Salazar, L. F. (2003). Sweet Potato Virus Disease (SPVD): Distribution, Incidence and Effete on Sweet Potato Yield in Peru. *Plants Dis*. **87:**297-302.

Haberle, S. G. (1998). Dating the Evidence for Prehistoric Agricultural Change in the Highlands of New Guinea: The last 2000 years. *Archaeol. Oceania* **47:**1–19.

Huaman, Z. (1996). Botany, Origin, Evolution and Biodiversity of the Sweet Potato. ***In:*** *Sweet potato Germless Management Training Manual* (CIP, 1996.).

Huang, J. C. and Sun, M. (2000). Genetic Diversity and Relationships of Sweet Potato and its Wild Relatives in *Ipomoea* series *Batatas* (*Convolvulaceae*) as Revealed by Inter-Simple Sequence Repeat (ISSR) and Restriction Analysis of Chloroplast DNA. *Theor. Appl. Genet.* **100:**1050–1060.

Ishak, J. A., Kreuze, J. F., Johansson, A., Mukasa, S. B., Tairo, F., Abo El-Abbas, F. M. and Valkonen, J. P. T. (2003). Some Molecular Characteristics of Three Viruses from SPVD-Affected Sweet Potato Plants in Egypt. *Arch. Virol.* **148:** 2449-2460.

James, W. C. (1974). Assessment of Plant Diseases and Losses. *Ann. Rev. Phytopath.* **12:**27-48.

Jarret, R. L., Gawel, N. and Whittemore, A. (1992). Phylogenetic Relationship of the Sweet Potato (*Ipomoea batatas* (L.) Lam.). *Am. Soc. Hort. Sci.* **117:**633–637.

Jordan, L. R., Hammond, J., Larsen, C. R. and Moyer, J.W. (1992). Use of Polyclonal Antisera and MonoclonalAntibodies to Examine Serological Relationships Among Three Filamentous Viruses of Sweet Potato. *Phytopathol.* **82:**713-717.

Kaitisha, G. and Gibson, R. W. (1999). Identification of Sweet Potato Feathery Mottle Virus and Sweet Potato Chlorotic Stunt Viruses in Sweet Potato in Zambia. ***In:*** *Food security and crop diversification in the SADC countries: The Role of Cassava and Sweet Potato*. pp. 293. (Okoroda, M. O. and Teri, L. M. eds.). *Proceedings of the Scientific Workshop of the Southern African Root Crops Research Network (SARRNET).* Lusaka, Zambia.

Kapinga R., Ortiz O., Ndunguru J., Omiat E. and Tumwegamire S. (2007). *Handbook of Sweet potato Integrated Crop Management: Research Outputs and Programs for East Africa.* International Potato Center (CIP). Uganda.

Karyeija, R. F., Gibson, R. W. and Valkonen, L. P. T. (1998a). Resistance to Sweet Potato Virus Disease (SPVD) in Wild East African *Ipomoea. Ann. Apple. Biol.* **133:**39-44.

Karyeija, R. F., Gibson, R. W. and Valkonen, L. P. T. (1998b). The Significance of Sweet Potato Feathery Mottle Virus in Subsistence Sweet Potato Production in Africa. *Plant Dis.* **82:**4-15.

Karyeija, R. F., Kreuze, I. F., Gibson, R. W. and Valkonen, L. P. T. (2000). Synergistic Interaction of a Potyvirus and a Phloem-Limited Crinivirus in Sweet Potato Plants. *Viro.* **26:** 26-36.

Karyeija, R. F., Kreuze, I. F., Gibson, RW. and Valkonen, L. P. T. (2001). Variability of Sweet Potato Feathery Mottle Virus in Africa. *Afri. Crop Sci.* **9:**293-299.

Kokkinos D. C. and Clark A. C. (2006). Interactions Among *Sweet Potato Chlorotic Stunt Virus* and Different Potyviruses and Potyvirus Strains Infecting Sweet potato in the United States. *Plant Dis.* **90:**1357-1352.

La Bonte, D. R. and Cannon, J. M. (1998). Production and Utilization of Sweet Potato in the United States. ***In:*** *Proceedings of International Workshop. Sweet Potato Production System Toward the 21st Century.* pp. 29–32 (La Bonte, D. R., Yamashita, M. and Mochida, H. eds.). Kyushu National Agricultural Station, Japan.

Laurie, S. M and Stork, P. O. (1997). A Scheme for the Distribution of Disease-Free Sweet Potato Propagation Material in South Africa. *Proceedings of the 4'th Triennial Congress of the African Potato Association.* Pretoria, South Africa, 23-28 February 1997.

Liu, H. Y, Wisler, G. C. and Duffus, J. E. (2000). Particle Lengths of Whitefly Transmitted Criniviruses. *Plant Dis.* **84:**803-805.

Loktrakul, P., Valverde, C. A, Clark, J.S. and De La Torre, R. (1998). Detection of Geminivirus Infecting Sweet Potato in the United States. *Plant Dis***. 82:**1253- 1257.

Luisa, H. and Robert J. H. (2000). A Geo-Referenced Database of Global Sweet potato Distribution ***In:*** *Production Systems and Natural Resource Management Department International Potato Center* (CIP). Working Paper No. 4. pp. 1-42.

Moyer, J. W. and Salaza, L. F. (1989). Virus Like Diseases of Sweet Potato. *Plant Dis*. **73:**451-455.

Mukasa, S. B., Rubaihayo, P. R. & Valkonen, J. P. T. (2003). Incidence of Viruses and Virus-like Diseases in Sweet Potato in Uganda. *Plant Dis.* **87:**336-340.

Mumford, R. A., Barker, I. and Wood, K. R. (1994). The Detection of Tomato Spotted Wilt Virus Using the Polymerase Chain Reaction. *J. Virol. Meth.* **46:**303-301.

Ndunguru J. and Kapinga R. (2007). Viruses and Virus-Like Diseases Affecting Sweet Potato Subsistence Farming in Southern Tanzania. *Afri. J. Agri. Res.* **2:**232-239.

Ndunguru J., Kapinga R., Sseruwagi P., Sayi B., Mwanga R., Tumwegamire S. and Rugutu C. (2009).Assessing the Sweet Potato Virus Disease and its Associated Vectors in Northwestern Tanzania and Central Uganda. *Afri. J. Agri. Res.* **4:**334-343.

Ngeve, J. M. and Bouwkamp, J. C. (1991). Effects of Sweet Potato Virus Disease (SPVD) on the Yield of Sweet Potato Genotypes in Cameroon. *Expla. Agri.* **27:** 221-225.

Nishiyama, I. (1971). Evaluation and domestication of sweet potato. *Bot. Mag. Tokyo.* **84:**377–387.

Njeruet, W. R.., Bagabe C.M., Nkezabahizi, D., Kayiranga D., Kajuga J., Butare L. and Ndirigue J. (2008). Viruses Infecting Sweet Potato in Rwanda: Occurrence and Distribution. *Ann. Appl. Biol.* **153**: 215–221.

Nyaboga, N. E., Ateka. M. E, and Bulimo D. W. (2008). Serological Detection of Virus Diseases of Sweet Potato in Kenya. *J. App. Biosci*., **7:**222–229.

Rossel, Gkriener, A, and Zjang, D.P. (2001). From Latin America to Oceania: The Historic Dispersal of Sweet Potato Re-examined using AFLP. In CIP program Report Lima, Peru.

Salazar, L. F. and Fuentes, S. (2000). Current Knowledge on Major Viruses of Sweet Potatoes. ***In:*** *International Workshop on Sweet Potato Cultivar Decline Study.* pp. 14. (Nakazawa, Y. and Ishiguro, K. eds.). Dept. of Upland Farming, Kyushu National Agricultural Experiment Station, Japan.

Sebsebe Demissew (2006). *Convolvulaceae* ***In:*** *Flora of Ethiopia.* pp 161-231. (Hedberge, I. and Edward, s. Ensermu Kelbessa, Edwards.S., Sebsebe Demissew ,Persson. E., eds.), *Gentianaceae* to *Cyclocheilaceae*. Addis Ababa/Uppsala, Ethiopia/Sweden, The National Herbarium, Vol. 5.

Singh, S. & Barker, H. (1991). Comparison of Penicillinase-based and Alkaline Phosphatase-Based Enzyme-Linked Immunosorbent Assay for Detection of Six potato Viruses. *Potato Res.* **34:**451-457.

SPL (Scientific Phytopatological Laboratory). (1986). Progress Report for the Period 1985/86, pp. 252-259. Ambo, Ethiopia.

Tameru Alemu (2004). Characterization of Viruses of Pepper (*Capsicum* spp.) and Sweet Potato (*Ipomea batatas*) from Ethiopia. Doctoral Dissertation. University of Bonn. pp. 126.

Tekalign Wondimu, Tileye Feyissa and Girma Bedada. (2009). Meristume Culture of Selected Sweet Potato (*Ipomoea batatas* (L.) Lam.) to Produce Virus Free Planting Materials. MSc. Thesis, Addis Ababa University, Faculty of Science.

Thompson, G. J.and Mynhardt, L. (1986). A Potyvirus Infecting Sweet Potatoes. *Phytophylact.* **18:** 46.

Thottapilly, G. and Rossel, H. W. (1988). Virus Disease of Sweet Potato in Nigeria, Improvement of Sweet Potato *(Ipomoea batatas)* in East Africa. Report of the Workshop on Sweet Potato Improvement in East Africa, ILRDA, Nairobi, Kenya, UNDP project CIAT-CIP-IITA, September, 28-October 2, 1987.

UN Population Fund (UNFPA). (2008). Ethiopian Editorial Statically Agency Population and Housing Census. Addis Ababa.

Untiveros M., Fuentes S., and Salazar F L. (2007). Synergistic Interaction of *Sweet Potato Chlorotic Stunt Virus* (*Crinivirus*) with Carla-, Cucumo-, Ipomo-, and Potyviruses Infecting Sweet Potato. *Plant Dis.* **91:**669- 676.

Valverde, R.A., Sim, J. and Lotrakul, P. (2004). Whitefly Transmission of Sweet Potato Virus. *Res*. **100:**123-128.

Van Bruggen, A. H. C. (1984). Sweet Potato Stem Blight Caused by *Alternaria* sp:A New Disease in Ethiopia. *Neth. J. Plant Pathol.* **90:**155-164.

Vincent, L. (2009). *Tropical Root and Tuber Crops: Cassava, Sweet Potato, Yams and Aroids.* MPG Biddles Ltd, King's Lynn, UK. pp 91-179.

Walkey, D. (1991). *Applied Plant Virology*. 2nd ed. Chapman and Hall, London.

Winter, S., Purac, A., Leggett, F., Frison, E. A., Rossell, H. W. and Hamilton, R. I. (1992). Partial Characterization and Molecular Cloning of a Closterovirus from Sweet Potato Infected with the Sweet Potato Virus Disease Complex from Nigeria. *Phytopathol.* **82:**869-875.

Yen, D. E. (1982). Sweet Potato in Historical Perspective. ***In:*** Sweet Potato, Proceedings of First Internal Symposium. pp. 17-30. (Villarreal, R.L. and Grigges, T.D. eds.) AVRDC publ. No. 82-172, Taiwan.

Zhang, D. P. and Li, X. Q. (2004). Sweet Potato as Animal Feed: The Perspective of Crop Improvement for Nutrition Quality. ***In:*** *Sweet Potato Postharvest Research and Development in China.* pp. 47–56. (Fuglie, K.O. and Hermann, M. eds). CIP, ESAP Region, Bogor, Indonesia.

Zhang, D. P., Cervantes, J., Huaman, Z., Carey, E. and Ghislain, M. (2000). Assessing Genetic Diversity of Sweet Potato (*Ipomoea batatas* (L.) Lam.) Cultivars from Tropical America using AFLP. *Genet. Res. Crop Evol.* **47:**659–665.

14. Appendices

Appendix 1: Nitrocellulose Membrane Enzyme-Linked Immunesorbent Assay (NCM-ELISA) Reagents

Tris-Saline Buffer (TBS)

Dissolve 0.02M Tris and 0.5M NaCl in1995ml distilled water and adjust pH to 7.5 by adding 5ml 18.5% HCl to the final volume of 2L.

Extraction Buffer

Dissolve 1gm of sodium sulfite in 500ml TBS

Blocking Buffer

Dissolve 12gm of Cow Milk in 600ml TBS to make 2%

Blocking Solution

Mix 6ml Triton X-100 in 300ml blocking buffer

Antibody Solution

Mix 30ml blocking buffer and Antibodies for SPFMV, SPVG, SPMMV, SPLV, SPCFV, SPCSV, C-6, SPCaLV, CMV and SPMSV in separate bakers

Conjugate Solution

Dissolve 1ml of Conjugate antibody, Goat Anti-Rabbit (GAR) in 300ml Conjugate buffer

Substrate Buffer

Dissolve 0.1M Tris, 0.1M NaCl and 5mM $MgCl_2$ d in 250ml distilled water and adjust pH to 9.5 by adding 0.5ml of 18.5% HCL.

Substrate Solution

Dissolve 25mg of Nitro-Blue Tetrazolium Chloride **(**NBT**)** in 1ml (70%) Dimethylformamide (DMF) and dissolve 12.5mg of 5-Bromo-4-Chloro-3'-Indolyphosphate

(BCIP in 1ml solvent (100%) Dimethylformamide (DMF) and mix both in 250ml of Substrate buffer

Washing Buffer

TBS+Tween20 1ml for 2L

Conjugate Buffer

Dissolve 6gm of Cow Milk in

300ml TBS to prepare 2%

Appendix 2: Triple Antibody Sandwich Enzyme-Linked Immunesorbent Assay (TAS-ELISA) Reagents

Coating Buffer

Dissolve in 900ml distilled water, adjust pH to 9.6 with HCl and make up to 1L

1.59g Sodium Carbonate Na_2CO_3

2.93g Sodium Bicarbonate $NaHCO_3$

PBS Phosphate Buffer Saline

Dissolve in 900ml distilled water, adjust pH to 7.4 with HCl and make up to 1L

8g Sodium Chloride NaCl

0.2g Monobasic Potassium Phosphate KH_2PO_4

1.15g Dibasic Sodium Phosphate Na_2HPO_4

0.2g Potassium Chloride KCl

PBS-Tween20 Washing

PBS+0.5ml Tween 20 per L

Extraction Buffer

PBST+2% PVP (PVP-15 polyvinyl pyrrolidone

Conjugate Buffer

PBST+2%PVP+0.2% bovine serum albumin

Substrate Buffer

Dissolve 97ml diethanolamine in 600ml H_2O pH at 9.8 make up to 1L

Dissolve 10mg p-nitrophenyl phosphate in 10ml Tris- substrate buffer

He who lacks understanding let him hear what wisdom cries into the ears of the fool. For the fool sayth in his heart there is no God.

The LORD is the God of heaven and earth.

If I am asked I will say unto thee…me is utterly helpless. See mine words if thou art keen to know what was in my heart.

"I feel like I have been hewing a rock for four years looking for gold which I have told locked inside. Alas, I got a ball of copper. Now…understand I am beguiled like Eve was. Yet, I learned how to break rocks… sweating I knew not … ask me about tears for my burned face will tell you a lot."

May the LORD of the living be blessed!

For whatsoever HE intendth to perform HE shallt.

Jubilee is mine friend now for I saw the mighty hands of the LORD.