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Survey and serological detection of sweet potato (*Ipomoea batatas* (L.) Lam.) viruses in Ethiopia

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ABSTRACT
Objective: The study aimed to assess the recent magnitude of virus diseases attacking sweet potato in the main production areas of the country.
Methodology and results: Ninety seven sweet potato fields were visited in eastern and southern Ethiopia in 2009 and a total of 235 symptomatic and 735 asymptomatic vine cuttings were collected. The vine cuttings were planted in an insect-proof screen house and tested for *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic fleck virus* (SPCFV), Sweet potato caulimo-like virus (SPCaLV), *Sweet potato mild speckling virus* (SPMSV), C-6 (flexious rod virus), *Sweet potato latent virus* (SwPLV), *Sweet potato virus G* (SPVG) and *Cucumber mosaic virus* (CMV) using Nitrocelullose Membrane ELISA (NCM-ELISA). Observations showed that the average prevalence of virus and virus-like symptom were 15.6% in Wolayita, 12.5% at Awassa (AARC), 10% in Hadiya, 6.3% in Gamo Gofa , 0.15% in Kembata-Tembaro, 0.1% in Sidama and 0.03% in East Hararge. The most prevalent virus was SPFMV (15.1%) followed by SPCSV (12.9 %) and SPVG (4.5 %). Mixed infection of SPFMV + SPCSV was the most common co-infection observed (9.3%) followed by SPVG+SPCSV (3%) of the total samples. Interestingly, no virus was detected in any of the samples obtained from Eastern and Western Hararge zones.
Conclusion and application of findings: This study has provided a quantitative assessment of both single and co-infecting viruses of sweet potato plants in farmer's fields in Ethiopia, and reveals the importance of selecting resistant varieties and production of virus free planting materials. Moreover, introducing internal quarantine would be important to minimize the virus movement from southern part of the country.
Key words: Sweet Potato, NCM-ELISA, Incidence, Virus, Single Infection, Mixed Infection

INTRODUCTION
Sweet potato (*Ipomoea batatas* (L.) Lam.) is a dicotyledonous plant which belongs to the family *Convolvulaceae*. It is an important tuberous root crop cultivated throughout the tropical and warm temperate regions wherever there is sufficient water to support growth (Austin, 1988; Demissew, 2006; Vincent, 2009). Sweet potato is a dry-land crop that is tolerant of a wide range of edaphic and climatic conditions. It is also 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). In Ethiopia, sweet potato is grown around a densely populated area in the South, Southwestern and Eastern parts of the country and is one of the most important crops for at least 20 million Ethiopians (Tofu *et al.*, 2007). In addition, it is cultivated in over 100 countries with over 98% of the production being consumed in developing countries. For every calorie consumed, sweet potatoes provide over 90% of essential nutrients except for protein and

niacin. For this reason it can be used as a seasonal staple when there is a shortage of other foodstuffs.

Plant pathogens including fungi, viruses and bacteria are responsible for increasing economic losses worldwide. Productivity of sweet potato is greatly constrained by pests and diseases that cause yield reduction by up to 98% (Kapinga *et al*., 2007). The most important one is viral diseases because the crop is sensitive to virus infection. African countries such as Nigeria and Uganda account for 50% yield loss. In East Africa, over 90% yield reductions have been associated with viruses (Cohen *et al.,* 1997; Gibson *et al*., 1998).

Twenty 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 reported to be the major viral disease in East Africa (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, and development of infectivity-based forecasting systems and control strategies (Chavi *et al.,* 1997; Moyer and Salazar, 1989). Currently, several sweet potato viruses have been identified and confirmed to be widely distributed in East Africa. These include two that belong to the family *Potyviridae*: *Sweet potato feathery mottle virus* (SPFMV, genus *Potyvirus*) and *Sweet potato mild mottle virus* (SPMMV, genus *Ipomovirus*); and the other two that belong to the family *Closteroviridae*: *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*) and *Sweet potato chlorotic fleck virus* (SPCFV, genus *Carlavirus*) (Gibson and Aritua, 2002; Mukasa *et al*., 2003).

Viruses have previously not 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 and proven by electron microscopy examination of sweet potato plants with mosaic symptoms from Nazreth. The virus was identified as SPFMV (SPL, 1986). Alemu (2004) reported a high incidence of SPFMV in some fields and the occurrence of SPVG mainly from Wolayita zone. Recently, a high level of virus incidence was reported in sweet potato germplasm resources maintained in research fields at Awassa and Wondo Genet in southern Ethiopia. The viruses were identified as SPFMV, SPCSV and Sweet potato virus II (Abraham, 2010). These previous studies have not covered most of the main sweet potato growing areas of the country being limited to few locations. This survey was therefore carried out to more comprehensively assess the current status of sweet potato viruses in farmer's fields in the major growing areas of Ethiopia.

MATERIALS AND METHODS

Field survey, sample collection and establishment: Farmers' fields were inspected in Southern and Eastern part of Ethiopia along roadsides at an average spacing distance of 6 km whenever the crop is grown (Fig.1). Five vine cuttings showing symptoms of a suspected viral infection and 5 symptomless (if any) from 2-4 month old sweet potato plants were randomly collected from each farmer's field. Samples were collected between 26th to 31st October (southern Ethiopian) and 27th of November to 1st of December, 2009 (eastern Ethiopia). A total of 970 (235 symptomatic and 753 asymptomatic) were collected during the survey. Virus disease incidence was estimated in each field by counting the proportion of symptomatic plants in a total of 100 plants according to James (1974).

Symptomatic and asymptomatic vine cuttings of sweet potato plants were kept in separate polyethylene bags labeled with the respective location and brought to Holetta Agricultural Research Center (HARC) for establishment. Vines from each sample were then planted in plastic pots containing sand: soil: cow dung in 3:2:1 ratios in insect proof screenhouse and watered every 2 days. Established plants were also inspected for the reproducibility of field symptoms and regularly sprayed to avoid potential spread by insect vectors between the plants.

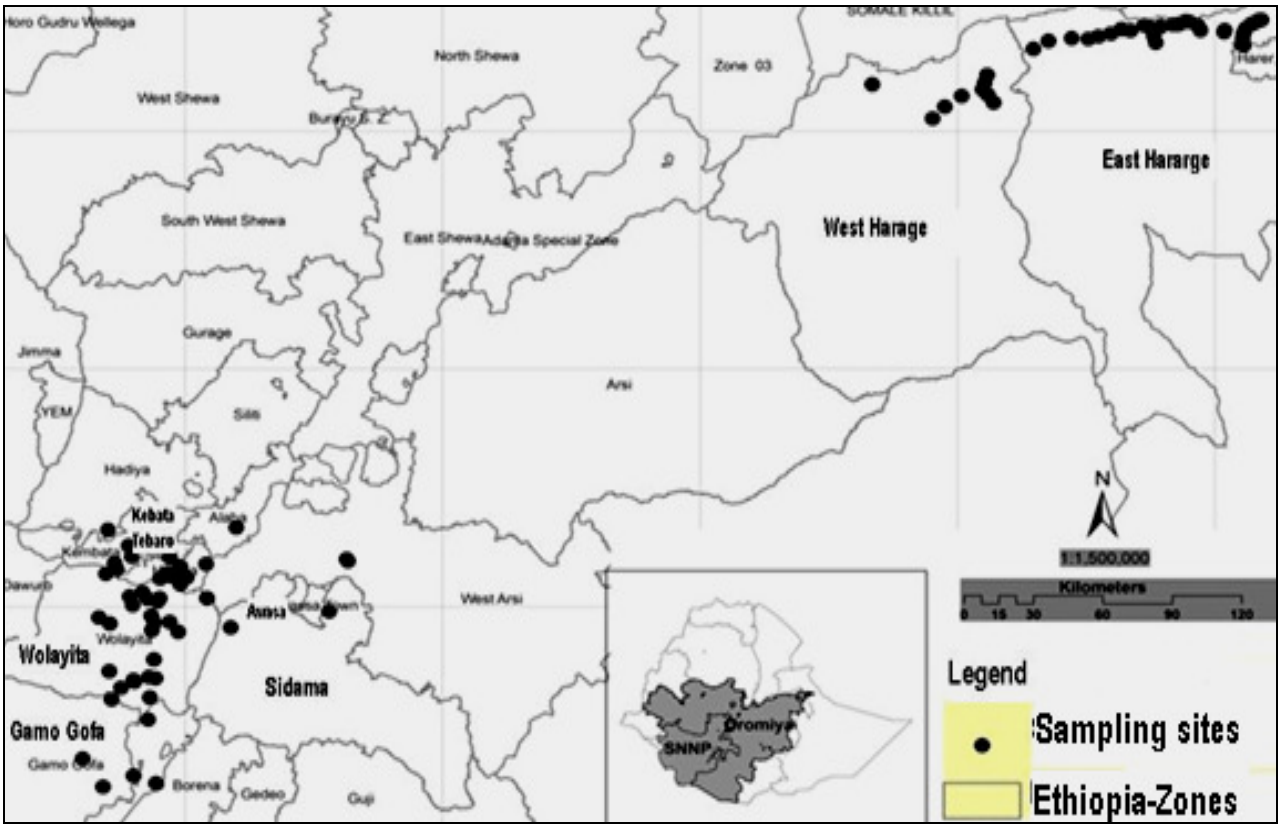

Figure 1: Map of Ethiopia showing the sites of sweet potato sample collection.

Serological identification of viruses: The nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) (Aritua *et al*., 1998) was conducted to identify the viruses using kits obtained from the International Potato Center (CIP, Lima, Peru). In the test, one centimeter diameter leaf discs were taken from a leaf on the top, middle and lower part of the stem from each plant. The sap extracted was spotted on NCM strip which was used along with those of virus-positive and non-infected control plants. Polyclonal antibodies specific to SPFMV, SPCSV, SPMMV, SPCFV, SPCaLV, C-6, SPMSV, CMV, SPVG and SwPLV were used for detection. The development of a purple color on the sample spots confirmed virus positive samples (Gutierrez *et al*., 2003).

RESULTS

The most commonly observed symptoms were general chlorosis, leaf clearing, leaf distortion, mosaic, purpling, stunting, 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 were inter-veinal chlorosis and general overall stunting of the plants (Fig. 2B and Fig 3). Plant samples that were only infected by SPVG 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 show vein chlorosis, yellow spotting, and leaf curling virus-like symptoms observed and (Fig.2 J) shows leaf from a healthy plant. Sero-negative sweet potato plants exhibited mild or no symptoms at all (Fig. 2J and 3).

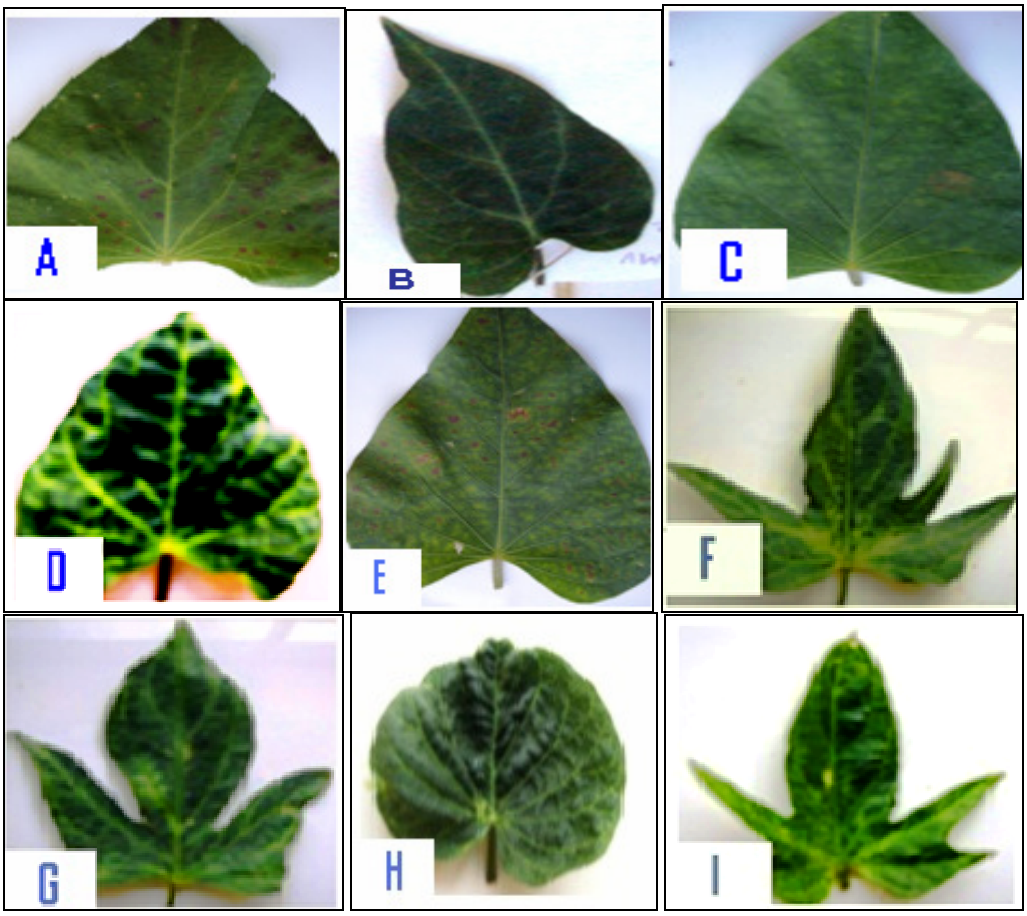

Figure 2: Detached leaves showing **v**irus-like and virus symptoms observed on sweet potato plants collected from Southern and Eastern part of Ethiopia. **A.** Purple spotted leaves of plants infected with *SPCSV*; **B.** Vein chlorosis leaves of plants infected with *SPFMV*; **C.** Chlorotic spotted leaves of plants infected with *SPVG*; **D.** Main vein chlorosis and distorted leaves of plants 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.

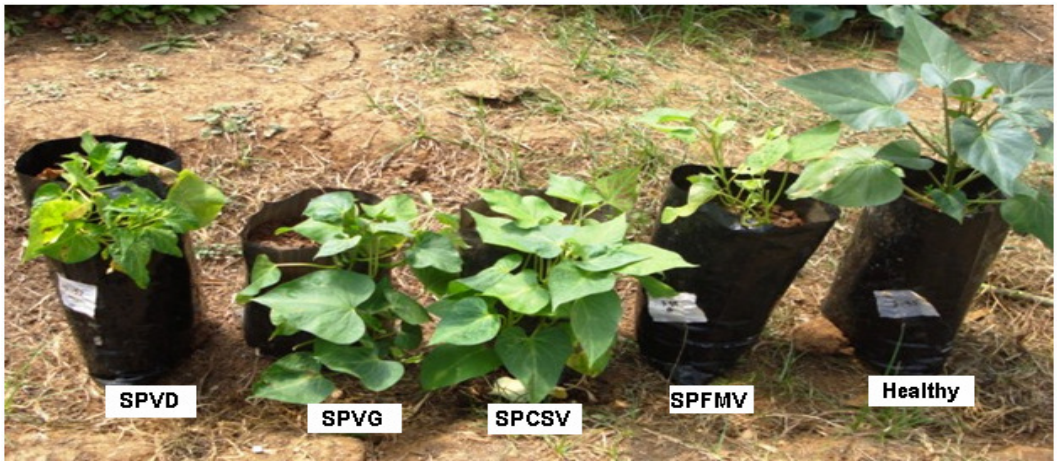

Figure 3: The degree of vigor between *SPVD, SPVG, SPCSV* and *SPFMV* infected sweet potato plants as compared to the healthy plant, picture taken in the screen house.

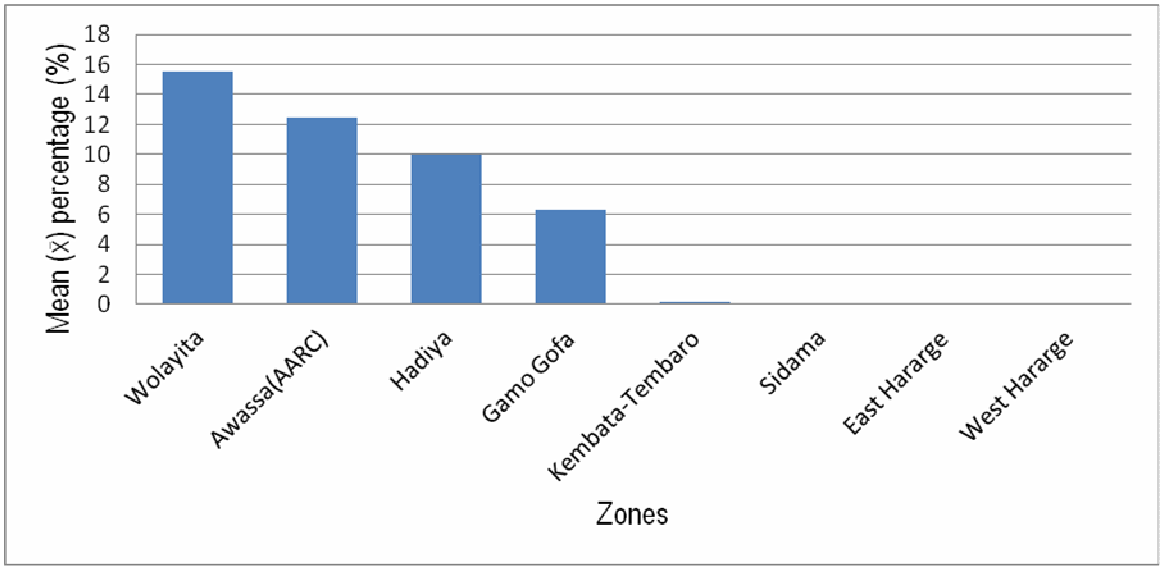

Figure 4: Mean (x□) prevalence (%) of virus and virus-like symptoms observed during the field survey.

The mean (x□) percentage of virus and virus-like symptoms was highest in Wolayita 15.55 %, followed by Awassa (AARC) 12.5 % then in Hadiya 10 % and it was highly decreased in Gamo Gofa 6.3 %, Kembata-Tembaro 0.15 %, Sidama 0.1 % and East Hararge 0.03 %. The incidence of symptomatic plants and observable symptoms drops to zero in Western Hararge (Fig. 4). An average of 20.7% of samples tested positive for at least one virus. Of the symptomatic samples, 68.5% of the samples reacted positive with antisera of one or more viruses with the frequency of detection being highest in samples from Hadiya and Kebata-Tembaro followed by Awassa, Wolayita and Gamo Gofa (Table 1). From asymptomatic sweet potato samples only 4.1% reacted positive with antisera of at least one virus. 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. Interestingly, none of the symptomatic and asymptomatic sweet potato plant samples from East and West Hararge were found to be infected with any of the viruses tested for (Table 1and 2).

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 eight zones in Ethiopia.

Zones	Asymptomatic Plants		Symptomatic Plant	
	Samples Assayed	Percentage (%)	Samples Assayed	Percentage (%)
Awassa (AARC)	10	30	10	90
Sidama	5	0	5	20
Hadiya	5	0	5	100
Wolayita	206	9.2	134	83.6
Gamo Gofa	96	8.3	34	67.6
Kembata-Tembaro	9	0	11	100
E. Hararge	314	0	36	0
W. Hararge	90	0	0	0
Total	735	4.1	235	68.5

Three sweet potato infecting viruses i.e. SPFMV, SPCSV and SPVG. were detected by NCM-ELISA in both symptomatic and asymptomatic sweet potato. However, none of the other viruses, SPMMV, SPCFV, SPLV, SPC-6, SPCaLV, SPMSV and CMV were detected (Table 2). As expected, the frequency of detection was higher in symptomatic than asymptomatic plant samples.

From the symptomatic samples, 133 (56.6%) and asymptomatic (13, 1.8%) reacted positive for SPFMV, indicating that SPFMV was the most prevalent virus. SPCSV was the second most frequent virus detected in 112 (48.9%) of the symptomatic and 10 (1.4%) asymptomatic samples, respectively. SPVG was third but occurred in low frequency being detected in 28 (11.9%) of the symptomatic and 16 (2.1%) of the asymptomatic plant samples (Table 2). Mixed infection of SPFMV and SPCSV was the most prevalent co-infection and was observed in 88 (37.4%) of the symptomatic plant samples and 2 (0.27%) of the asymptomatic plant samples. The second most prevalent dual infection was SPCSV+ SPVG in 7 (0.9%) of the asymptomatic and 25 (10.2%) of the symptomatic samples (Fig. 7).

Figure 7: Prevalence of single and dual virus infections detected using NCM-ELISA assay in sweet potato samples obtained from 97 fields.

The frequency of detection of virus infections varied with sampling zone (Table 1). Of the eight sampling zones the frequency of detection of SPFMV was very high in five zones of Hadiya 5 (100%) followed by Kembat-Tembaro 11 (100%), Awassa (AARC) 7 (70%), Gamo Gofa 23 (67.6%) and Wolayita 86 (64.7%) of the symptomatic plant samples. From the asymptomatic plant samples Gamo Gofa was the highest with 6

samples (6.3%) followed by Wolayita with 7 samples (3.4 %.)

Of the symptomatic plant samples SPCSV was detected at lower rates than SPFMV with a frequency of 5 (100%), 10 (90.9%), 91 (67.9%), 8 (23.5%) and 1 (10%) in Hadiya, Kembata-Tembaro, Wolayita, Gamo Gofa and Awassa zones, respectively. SPCSV was detected in fewer asymptomatic plant samples obtained from Wolayita (7 = 3.4%) and Gamo Gofa (3 =3.1%). The frequency of detection of SPVG was even lower than the other two viruses and was found at only three locations Awassa 2 (20%) and 3 (30%), Wolayita 24 (17.9%) and 11 (5.3%) and Gamo Gofa 3 (5.9%) and 2 (2.1%) of the symptomatic and asymptomatic plant samples, respectively (Table 2). SPVG was 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). Single and Mixed Infections: The incidence of mixed and single infection varied as shown in Figures 8 and 9, in each zones. From the asymptomatic plant samples Gamo Gofa with 5 samples (5.2%) was the highest followed by Awassa with 6 samples (2.9%) while from the symptomatic plant samples Gamgofa had 23 samples (67.6%), Awassa 6 samples (60%), Sidama 1 sample (20%), Wolayita 17 samples (12.7%) and Kebata-Tembaro 1 sample (9.1%) that reacted positive for SPFMV. The frequency of latent single SPVG infection was higher at Awassa with 3 samples (30%) followed by Wolayita with 3 samples (1.5%).

Symptomatic plant samples which were sero-positive for only SPVG were 2 (20%) and 4 (3%) in Gamo Gofa and Awassa zones, respectively. In Wolayita 1 (0.5%) asymptomatic and 2 (1.5%) symptomatic, and in Gamo Gofa 3 (8.8%) symptomatic samples were detected being infected by SPCSV alone.

Furthermore, mixed infections observed were SPFMV+SPCSV and SPCSV+SPVG (Fig.9). Incidences of SPFMV+SPCSV infection in the symptomatic plant samples were high in Hadiya with 5 samples (100%) followed by Wolayita with 69 samples (51.1%), Kembata-Tembaro 10 samples (50%), Awassa 1 sample (10%) and Gamo Gofa 3 samples (8.8%). Latent infection of SPFMV + SPCSV was observed in 1 sample (0.5%) obtained from Wolayita and 1 sample (1.04%) from Gamo Gofa.

Table 2: Proportion of symptomatic (S) and asymptomatic (A) sweet potato plant samples from the eight zones of Ethiopia that reacted positive for different viruses.

Zones	Plants Assayed		Percentage (%) SPFMV		Percentage (%) SPCSV		Percentage (%) SPVG	
	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
Kembata-Tembaro	11	9	100	0	90.9	0	0	0
E.Hararge	36	314	0	0	0	0	0	0
W.Hararge	90	0	0	0	0	0	0	0
Total	235	735	56.8	1.8	48.9	1.4	11.9	2.1

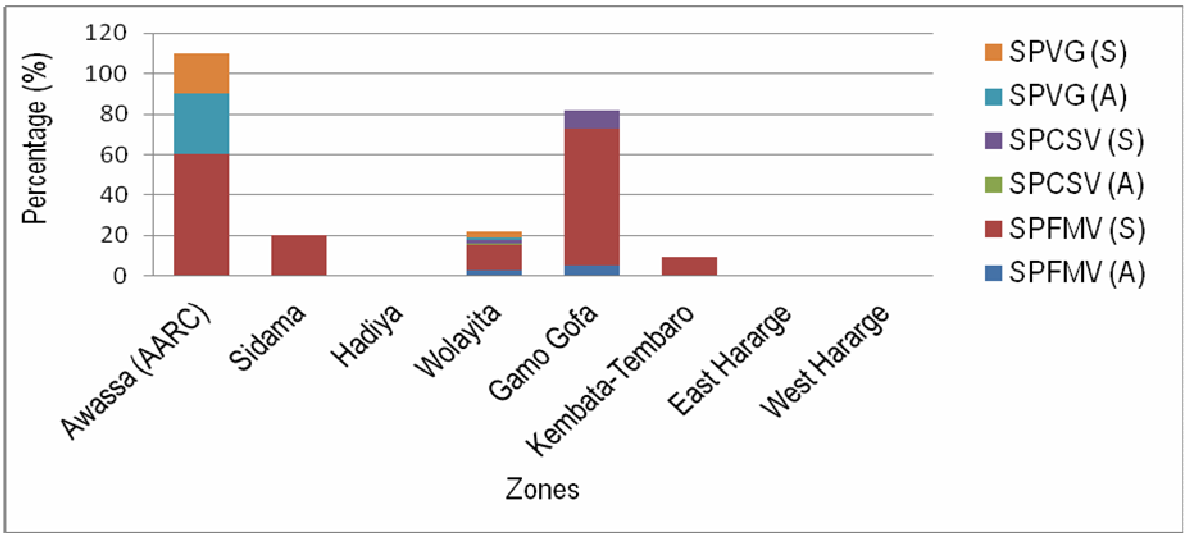

Figure 8: Proportion of asymptomatic (A) and symptomatic (S) single virus infections detected by NCM-ELISA assay from the eight zones of Ethiopia.

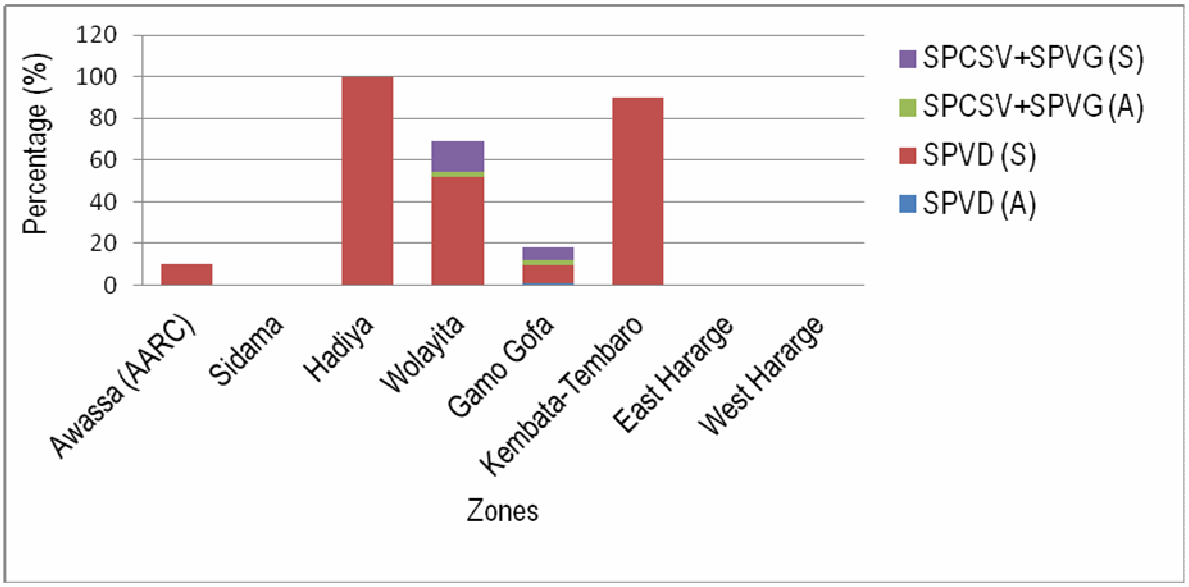

Figure 9: Proportion of asymptomatic (A) and symptomatic (S) mixed virus infections detected by NCM-ELISA assay from the eight zones of Ethiopia and *in vitro* plantlets.

The second type of mixed infection observed was SPVG+SPCSV and its frequency in Wolayita 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 Wolayita and Gamo Gofa. Incidence of SPCSV+SPVG infection from the asymptomatic plant samples was higher when compared to SPVD.

DISCUSSION

The results of the current study revealed a high prevalence of virus diseases in farmers' fields in southern Ethiopia and a low prevalence in Eastern Ethiopia. In recent years, there have been reports of increasing importance of sweet potato virus diseases. Alemu (2004) reported a high incidence of SPFMV in some fields and the occurrence of another virus named SPVG mainly from Wolayita and Awassa areas. However, the report concluded that the absent SPVD in the country was SPCSV. On the other hand, Abraham

(2010) reported a high prevalence of both SPFMV and SPCSV in research fields at Awassa and Wondo Genet. This survey is the first to be carried out in all the main sweet potato growing zones of the country. The results indicate that both SPFMV and SPCSV, the component of devastating SPVD, are prevalent in most zones of southern Ethiopia. The symptoms observed in the fields clearly resemble those of SPVD.

Three viruses namely SPFMV, SPCSV and SPVG were detected in sweet potato plants collected from farmers' fields in the main growing areas of Ethiopia. SPFMV is the most widespread followed by SPCSV. These two viruses are the most common and damaging as reported in other East African countries like Uganda, Kenya and Tanzania (Mukasa *et al*., 2003; Ateka et al., 2004). Hence, any future management attempts should concentrate on these two viruses.

The other seven viruses included in the tests were not detected in any sample. This result is in agreement with Alemu (2004) who did similar tests and did not detect these viruses either. Of these viruses, SPMMV and SPCFV were detected in Uganda and Kenya although at low prevalence. It is possible that these viruses either do not exist in the country or are rare that they escaped sampling.

Virus disease prevalence was generally higher in southern Ethiopia (e.g. Wolayita zone) and very low in Eastern Ethiopia. Compared to the cooler, wet, higher altitude areas of Eastern Ethiopia, Southern Ethiopia is in the lower altitude, warmer and drier climate which may have favored a higher population of the aphid and whitefly vectors of the viruses thereby resulting in higher disease incidence as suggested by Aritua *et al*. (1998).

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, very severe symptoms appear. This is equivalent to previous reports from, USA (Kokkinos and Clark, 2006) and East Africa regions: Tanzania, Ethiopia, Kenya and Uganda (Gibson *et al*., 1998; Mukasa *et al*., 2003; Alemu, 2004; Ndunguru and Kapinga, 2007; Nyaboga *et al*., 2008; Ndunguru *et al*., 2009). In this study, SPCSV occurred most frequently in mixed infections with SPFMV than alone, which agrees with previous 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, South Africa and other countries in Eastern Africa such as Ethiopia and Kenya (Ishak *et al*., 2003; Alemu, 2004; Kokkinos and Clark, 2006). No virus was detected in 15.3 % of the symptomatic plants, which clearly look diseased. It is not clear whether these symptoms are due to the existence of other viruses or genetic abnormalities resulting in similar diseases.

On the other hand, 4.1 % of the non symptomatic plants had latent virus infection. Since these plants resembled healthy ones, farmers may not be able to distinguish and exclude such infected cuttings from the planting materials they select for the next crop, thus contributing to spread of these viruses. Mixed virus infections in sweet potato are a common phenomenon as has been reported by Gibson *et al*. (1998). 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 agrees 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 (Njeru *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 prevalence of virus disease in sweet potato fields in southern Ethiopia suggests that the disease has the potential to undermine food security in the areas. This calls for the concerned bodies to work towards providing farmers with virus-free planting materials originating from tissue culture and grown in higher altitude areas that are free of insect vectors. Similarly, there is a need for introducing internal quarantine to minimize the movement of virus-infected sweet potato materials from highly infected areas of southern parts of the country to other places like Eastern Ethiopia.

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