Intensity of *Sweetpotato feathery mottle virus* and *Sweetpotato chlorotic stunt virus* in Farmers and Commercial vine Propagators Fields in Selected Areas of Southern Ethiopia

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Abstract

In Ethiopia, the production of sweet potato suffers from virus infections over the last two decades. To this effect, field surveys were conducted to identify and document the current incidences and severities of sweet potato viruses in farmers and commercial vine propagator fields in selected areas of Southern Ethiopia. In total, 710 leaf samples were collected from randomly selected 30 small-scale farmers' sweet potato fields, four commercial sweet potato vine propagators and one-government research institution during 2017. The selected plants were visually examined and disease severity was recorded based on 1-5 scales. Sweet potato feathery mottle virus (SPFMV) and Sweet potato chlorotic stunt virus (SPCSV), the two most common viruses were tested using DAS-ELISA and TAS-ELISA in the Plant Tissue Culture Laboratory at College of Agriculture, Hawassa University. This study revealed that sweet potato crops in farmers and vine propagator fields were infected by both viruses. The highest average incidences of SPCSV (59.1%) and SPFMV (37%) were recorded from farmers' fields in Boloso Sore district. The average incidences of SPFMV were 28% and 39.3% in the farmers' fields and commercial vine propagators fields, respectively, compared to 47% and 36.6% for SPCSV in the same fields. Overall, incidences of 38.2% and 37.6% were recorded for SPFMV and SPCSV, respectively, from all samples collected from studied areas. The mean highest virus severity of 3.03 and 2.97% was recorded in the commercial vine propagator and farmers' fields, respectively. This study revealed an increasing incidence and severity of the two viruses in the surveyed areas indicating the importance of planning for possible virus management and restrictions that limit further propagation of the planting materials from these areas to other locations where there were no reports of these viruses.

Keywords: incidences, infection, farmers, sweet potato, vine propagators, viruses

Introduction

Viruses are important pathogens infecting sweet potato worldwide. To date more than 30 sweet potato infecting viruses are identified and described in the world (Clark *et al.*, 2012). Previous reports have shown that sweet potato virus disease (SPVD) causes 56-98% yield losses, hence it is very critical to the production of sweet potato in Africa (Geddes, 1990; Gibson *et al.*, 1998).

Viral diseases are the second limiting factor of sweet potato production in Ethiopia after insect pests. To date more than eight sweet potato viruses are reported in Ethiopia (Dereje Haile *et al*., 2020a). *Sweet potato feathery mottle virus* (SPFMV) and *Sw*eet *potato chlorotic stunt virus* (SPCSV) are the two most commonly detected viruses (Dereje Haile *et al*., 2020a). *Sweet potato feathery mottle virus* was reported for the first time in 1986 (Scientific Phytopathological Laboratory, 1986) and later by many authors (Tamiru Alemu, 2004; Tesfaye *et al.*, 2011; Adane Abraham, 2010; Dereje Haile *et al.*, 2020b). SPCSV was reported for the first time by Adane Abraham (2010). These viruses were reported as single and mixed infections in Southern Ethiopia (Tesfaye *et al.*, 2011; Adane Abraham, 2010; Dereje Haile *et al.*, 2020b).

Ethiopian farmers have no adequate supply of certified virus free sweet potato planting materials. Farmers obtain sweet potato planting material from two sources/seed systems in Ethiopia. The first and main source is seeds saved from previous harvesting season and use it for next planting season. Such continuous use of own planting material for many seasons enhances the disseminations of viruses and resulted in accumulation of viral load over season. The second is a seed system where National Sweet Potato Research Program provides virus tested basic planting materials to few commercial; private fields and organized cooperative association for further multiplication for business and distribute through governmental and non-governmental organization (NGOs) to farmers who live in vulnerable conditions. The later source supplies very little planting material to the growers. Moreover, the national sweet potato germplasm collections maintained at Hawassa Agricultural Research Center (HARC) are mostly contaminated with virus infections (Tesfaye Tadesse *et al.*, 2013; Adane Abraham, 2010). Hence, exchange of materials between regions and countries are potential virus dissemination method.

Recently, six new viruses of sweet potato that were not previously reported from Ethiopia has been detected in newly introduced sweet potato plants that were maintained at National Collections Site at Hawassa Agricultural Research Center (Shiferaw Mokkonnen *et al.*, 2017). According to the authors, these new viruses were possibly introduced from abroad along with the planting materials imported and used for trails. The introduction of new viruses into country will further bring problem to sweet potato production. In previous study, farmers expressed that yields they obtain has been declining and production is under threat in Ethiopia by both pathogens and insect pests (Dereje Haile, 2019). In addition, the decline in productivity is also seen from FAO data (FAO, 2017).

Moreover, currently sweet potato is being promoted to drought prone areas in Ethiopia. If planting materials used for this purpose are infected by viruses, they become potential infection sources and devastating to farmers local cultivar in the new areas. Therefore, information on recent infection status of the two most common viruses in the farmers' fields and commercial multipliers' fields are important to plan for possible management and restriction that limit further propagation and its introduction to new areas. However, studies conducted on sweet potato infecting viruses in Ethiopia are limited. This is confirmed by the few numbers of virus's surveys conducted between 1986 to 2017 in Ethiopia (Dereje Haile *et al.*, 2020a). The present study was thus conducted to document the current information on the incidence of SPFMV, SPCSV, and their coinfection in the farmers' and four commercial sweet potato vine propagators fields in Southern Ethiopia.

Materials and Methods

This virus survey was conducted in Southern Ethiopia during the year 2017. Virus testing was carried out in the Plant Tissue Culture Laboratory at Hawassa University, College of Agriculture. Antibodies for the test were obtained from Leibniz institutes DSMZ-German Collection of Microorganism and Cell Culture, Germany through the facilitation of Norwegian University of Life Sciences with the support of NORHED project.

Field and leaf sampling

The survey included 30 small-scale farmers' sweet potato fields in two districts, four private commercial vine propagators and one-government research institution working on sweet potato in Southern Ethiopia. The districts were selected based on volume of sweet potato production and previous virus survey reports. Three kebeles (lower administrative unit) in each district, five farmers' fields in each kebele were randomly selected and leaf samples of five symptomatic and two symptomless plants in each field were collected from sweetpotato crops of 2 - 4 months old along two diagonal transects. This means about 210 leaf samples were collected from 30 small-scale farmers' sweet potato fields. In addition, leaf samples from 500 randomly selected plants were collected from 4 private vine propagators and one government institution propagating sweet potato. In total, 710 plants were randomly selected, and samples were collected from each plant for testing targeted viruses. The selected plants were examined for the presences of virus-like symptoms and the symptom severity level of each plant was recorded according to the standard set by (Ndunguru *et al.*, 2009). Briefly, symptom severity was assessed visually using a scoring scale of 1 - 5 where 1 = symptomless plant and 5 = most severe symptoms including leaf distortion, stunting of plants, clear vine clearing of plants (Ndunguru *et al.,* 2009).

Laboratory testing

The two most economically important viruses; SPFMV and SPCSV, previously reported in the region, were assessed in this survey to find out their current level of infection. Leaf samples were tested for SPFMV and SPCSV using DAS-ELISA and TAS-ELISA procedures (Clark and Adams, 1977; Abraham *et al.,* 2006), respectively, with minor modification. Antisera for the viruses were obtained from the Leibniz institutes DSMZ-German Collection of Microorganism and Cell Culture, Germany. All the antibodies were diluted following dilution ratio indicated in the manufacturer recommendations. Coating antibody and detection antibody were diluted in 1:1000 ratio in a coating buffer and conjugate buffers, respectively. Subsequently, 100µl of each antibody was added into duplicate well of ELISA plate which is modified from the 200 µl recommended in the protocol. All the incubation steps were done at 37 ^{0}C for 3 hours, except the overnight incubation at +4 ^{0}C after sample additions. Sap was extracted from 0.5 g leaf sample using sample extraction buffer (phosphate buffered saline plus tween 20 + 2% PVP). Positive and negative controls corresponding to each virus were added in duplicate wells and samples that showed clear yellow color in the duplicate wells, within two hours after substrate addition, were considered as virus infected samples.

Data analysis

The percentages of infected samples/incidences percentages were calculated out of the total number of samples tested, for each kebele, district, and commercial fields and presented in graphs. Symptom severity was assessed visually using a scoring scale of 1 - 5 where 1 = symptomless plant and 5 = most severe symptoms including leaf distortion, stunting of plants, clear vine clearing of plants (Ndunguru *et al.,* 2009). The mean symptoms severity was calculated for each plant in the farmer field and commercial vine propagators.

Results and Discussion

Field observation

Sweet potato virus-like disease symptoms were observed in all the surveyed areas and were highly variable among locations, fields and varieties. None of the farmers' and private vine propagators' sweet potato fields surveyed were free of symptoms. In some fields, we have observed patches of symptomatic plants due to severe infection and others have relatively light infection distributed all over the farm (Fig.1C). Some of the observed symptoms in the field include vein clearing, feathery mottles, leaf deformation, and discoloration, stunting of plants and yellowing of leaves (Fig.1 A-B). However, filed with no symptomatic plants does not mean the farm is free of viruses, as some viruses cause only mild or latent infection in plants (Brunt *et al.*, 1996). It is suggested that symptoms with clear virus-symptoms have to be removed from the farm to limit any possible spread of the pathogens casing the diseases and associated symptoms.

Fig.1 Farmer sweet potato field in Boloso Sore district, Wolayta zone, Ethiopia. Fig. A: sweet potato with vein clearing and mosaic symptoms; Fig. B: virus infected stunted plants with narrow leaves; Fig. C: 'Kulfo' plants that are healthy looking (left) and with narrow leaves showing infections (right).

Incidences and severity of viruses in the surveyed areas

Results revealed SPFMV and SPCSV were detected as single and mixed infections in the farmers' and commercial vine propagators' fields located in study areas in Southern Ethiopia (Fig. 2 and 4, Table 2). Incidences of SPFMV and SPCSV varied between location (districts and kebeles') and between farmers' fields', private commercial vine propagators and among plants sampled within a given field (Fig. 2 and 3, Table 3).

Incidences of SPCSV and SPFMV in farmers' fields

The highest (59.1% and 36.2%) district average incidences of SPCSV were detected in Boloso Sore and Sodo Zuria districts, respectively (Fig. 2). SPCSV have been reported previously in Southern Ethiopia by Tewodros Tesfaye *et al.* (2011), Adane Abraham (2010) and Dereje Haile *et al.* (2020b). The highest (77.1%) and the least (25.7%) incidences of SPCSV infections at village were obtained from Wormuma and Doyo Weibo kebeles/villages in Boloso Sore district, in that order (Fig 3). Compared to incidence of SPCSV (12.9%) in previous viruses survey in similar areas in Ethiopia (Tewodros Tesfaye *et al.*, 2011), this survey highlighted an increasing average incidence of SPCSV (47.1%) in the farmers' fields. A higher incidence of SPCSV was detected not only in the farmers' fields, but also, in the commercial vine propagators fields that were sampled and tested. Besides, there exist high numbers of SPCSV positive reaction than SPFMV in the tested samples collected from farmer's field in almost all areas, indicating SPCSV is spreading in Southern Ethiopia faster than before. This increases in the percentage of SPCSV incidences overtime explains the severe symptoms observed in the fields (Fig 1). The increasing SPCSV infection in sweet potato cause significant losses in agricultural sweet potato crops worldwide, affecting the yield and quality of agricultural products. This virus is white fly transmitted; controlling the white flies in the filed can reduce further transmission and spread of the virus.

Like SPCSV, the highest average incidence of SPFMV infection (34.3%) was also recorded in Boloso Sore district (Fig. 2). The average incidence of SPCSV is higher than that of SPFMV indicating that a greater number of fields were infected by SPCSV in studied locations. In this district, the highest and the least SPFMV incidences of 65.71% and 8.6%, respectively were detected in Gurmu Kosha and Doyo Weyibo kebeles of Boloso Sore district (Fig. 3). The average incidence of SPFMV in the present survey was much higher than what had been reported (15.1%) in previous surveys conducted in the same areas (Tewodros Tesfaye *et al*., 2011).

Mixed infection of SPCSV and SPFMV, often causing SPVD, was detected in both districts; the highest incidence (29.5%) in Boloso Sore and the least (9.5%) in Sodo Zuria (Fig 2). Among the kebele, the highest (62.9%) and the least (2.9%) incidence of this mixed infection were detected in the leaf samples collected from farmers' field in Gurmo Kosh and Doyo Weibo, respectively (Fig. 3). Previous study had reported an incidence of 9.3% for mixed infection of SPCSV and SPFMV (SPVD), which is less than the average incidence (19.5%), recorded in this study.

An interesting aspect was the virus like record observed in the field during samples collection was 90% in accordance with the symptom severity, meaning those plants with virus-like symptoms were at least infected by one of the viruses it tested for. Even though there were differences in the level of the incidences; none of the farmers' field and private commercial vine multiplication site were free of infections (Tables 2 and 3), indicating the necessity to train farmers and commercial vine propagators on how to reduce the sources of infection and maintain the health of the plants.

The present finding is in agreement with previous studies that indicate the level of virus infection/incidence varies with the type of the virus and locations. For instances, SPFMV infections reaching 100% incidence or close to it in USA (Bryan *et al.*, 2003; Clark and Hoy, 2006), heavy infection of SPFMV, SPCSV and mixed infection (SPFMV + SPCSV) in Israel (Milgram *et al.*, 1996) and widespread occurrence of SPFMV with no mixed infections with SPCSV in Australia (Maina *et al.*, 2018) were reported. However, the lower detection of SPFMV in the present study might be linked to the nature of the virus that it accumulates lower virus titters in infected plants particularly under a condition of single infection, in which SPFMV causes rather mild mottling or no symptoms in sweetpotato cultivars (Karyeija *et al.,* 1998). Studies reported that the titer of SPFMV in plant tissue infected with SPFMV alone is low and can increase by up to 600-fold when co-infected with SPCSV (Karyeija *et al*., 2000; Tairo *et al*., 2005). This is of high concern in vegetative propagated plants as latent infection usually symptomless, looks health and exposed to lesser chance of deselection and can be further used as planting materials and spread the virus. The presence of infection in such materials mostly reveals after graft inoculation and testing always should involve grafting before ELISA test in verification of such planting materials. Moreover, the varieties tested in the present study were only two, whereas previously studies tested many cultivars in the farmers' fields and collection in the research sites which include SPFMV susceptible cultivars. Nonetheless, the present study showed SPFMV and SPCSV, are more widely distributed than it was previously recognized.

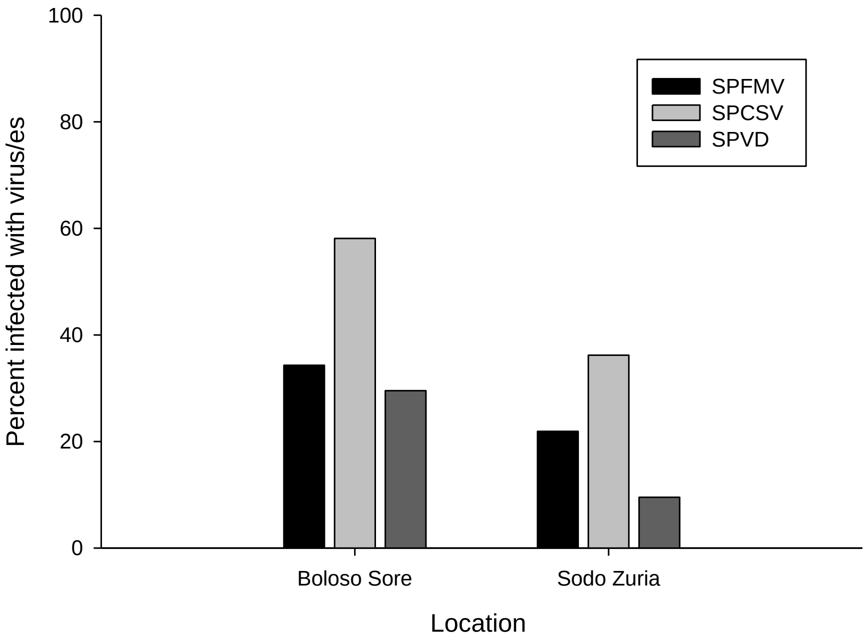

Figure 2. Incidences of sweet potato viruses in the farmers' fields at the district level

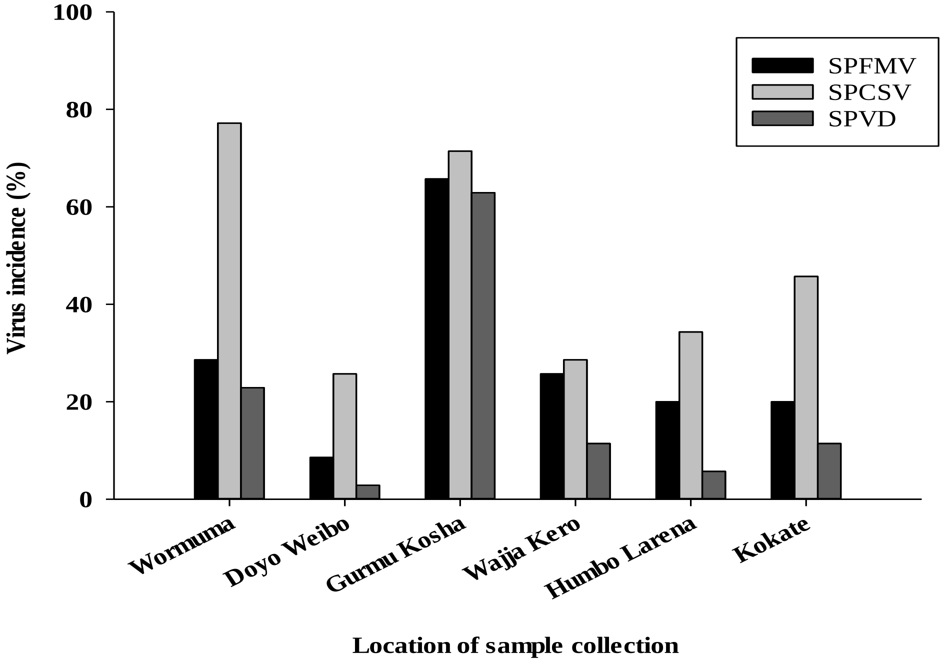

Figure 3. Incidences of sweet potato viruses in the farmers' fields at kebele level

Mean severity of symptom in vine propagators fields

The symptom severity scores varied between the surveyed locations. The maximum (3.03) and minimum (2.55) severity scores were recorded from the private sweet potato vine propagators fields at Bilate and Wolayta, respectively (Table 1). The mean maximum severity score does not necessarily mean high incidences of the infecting viruses. For example, our result showed that even though the mean severity score is highest in Bilate Jara Farm, the mean highest incidence is scored in Wolayta area farm (Fig 4). This might indicate that virus like disease symptoms does not necessarily mean the plant is virus infected rather it could also be appears due to other factors like environment (moisture stress, nutrient shortage) where the crops grow and other disease causing pathogens.

Table 1. Mean severity score of virus-like symptoms collected from sweet potato fields in Wolayta zone and Sidama region.

Area surveyed	Owners	No of samples	Mean severity score
Boloso Sore district	Farmers	105	2.97
Sodo Zuria district	Farmers	105	2.66
Lambadina	Vine propagators	90	Not assessed
Jara Farm (Bilate)	"	110	3.03
Leku (Sidam Region)	"	90	2.78
HARC	"	90	Not assessed
Wolayta	"	45	2.55

Table 2. Presence of sweet potato infecting viruses in six kebeles of Wolyta zone, SNNPR

Kebeles	Viruses		
	FMV	CSV	SPFMV + SPCSV
Humbo Larena	+	+	+
Delbo Wogene	+	+	+
Waja Kero	+	+	+
Gurmo Kosha	+	+	+
Doyo Weyibo	+	+	+
Wormuma	+	+	+

*Virus testing was conducted based on 35 samples collected from each kebele

Incidences of SPCSV and SPFMV in commercial sweet potato vine propagators fields

One or two of the viruses were detected in 71.1%, 65.5%, 60%, 46.2% and 53.3% of leaf samples tested from commercial vine propagators fields located in Wolayta, Lambadina, Bilate, Leku and National Sweet Potato Collections of HARC, respectively (Table 3, Fig. 4). The highest incidence of SPFMV (62.2%) in private vine propagator fields was registered in Lambadina areas of Arbamich zone while the least incidence (27.7%) was recorded in Jara Farm at Bilate, Wolayta zone. Similarly, the highest incidence of SPCSV (58%) was observed in vine propagator farm in Wolayta zone and the least (26.15%) was from Jara farm.

The highest (53%) incidence of SPVD in vine propagators fields was recorded at Lambadina, Arba Minch zone whereas the least (1.7%) was from Leku, Sidama region. Unlike what was observed in the farmers' fields, SPFMV had the higher average percentage incidences (39%) at the private vine propagators' sites than the average incidence of SPCSV (36.1%) and SPVD (15.4%).

The infection observed in field-grown mother stock and net tunnels samples obtained from research center suggests that virus infection already started during the multiplication of basic seeds and this virus infection are further propagate during field multiplication of commercial planting materials. Hence, research institution and seed-vine multiplication companies should screen their materials for viral pathogens before distributing to farmers. On the other hand, no symptom on a plant does not mean that the plant is free of viruses. This is because some viruses cause only mild or no symptoms in plants (Brunt *et al.*, 1996). Rouging diseased plants at early stage removes infection sources and planting new crops isolated from older ones have been shown experimentally to reduce the virus spread considerably (Gibson *et al.*, 2003).

Mixed infection

SPVD, caused by mixed infections of SPFMV and SPCSV has been previously reported from Ethiopia (Adane Abraham, 2010; Tewodros Tesfaye *et al.*, 2011). Results of the present study also revealed existence of SPVD at high incidences in the farmers, vine propagators and National collection site at Hawassa, indicating the disease incidence is increasing over time and is a high threat to sweet potato production in Ethiopia. For instance, the incidence of SPVD was 3.9 - 37.4% in the previous years (Adane Abraham, 2010, Tewodros Tesfaye *et al*., 2011). However, in this study the disease incidence reaching up to 59% was registered in Boloso Sore. SPVD is a very common devastating disease limiting sweet potato production in other Sub-Saharan Africa (Geddes, 1990; Gibson and Aritua, 2002). Co-infection of SPCSV with SPFMV reported to cause severe yield losses reaching as high as 90% (Loebenstein, 2015). The detection and wide distribution SPFMV, SPCSV in single and mixed infections depicts the threat of SPVD on sustainable sweetpotato production and seed system in Ethiopia.

Level of virus/es incidences varied between varieties

Virus incidence level varied for different sweet potato varieties, showing the differential response to viruses causing SPVD (Table 3). For instance, 'Kulfo' is more affected by SPFMV than 'Hawassa 83' at all locations. Similarly, at some fields, 'Kulfo' is more affected by SPCSV than Hawassa 83 when grown in the same field. The highest SPFMV incidence (77.7%) for variety 'Kulfo 'was recorded from private propagator farm at Lambadina followed by the field at Bilate (53.85%). This study revealed Kulfo, an Orange fleshed sweet potato move infected than Hawassa-83, the white fleshed sweet potato. This may indicate the existence of natural differences in resistance to pathogen between cultivars and host preference of vectors transmitting the viruses. Exploiting the natural difference in resistance to diseases among cultivars is vital through careful selection of unaffected plants as sources of cuttings for new plantings, identify and use gene(s) conferring resistance are possible options for virus disease management (Mwanga *et al.*, 2002). The best example is SPVD resistant sweet potato varieties (NASPOT 1 to 6) are released from Uganda (Mwanga *et al.*, 2003).

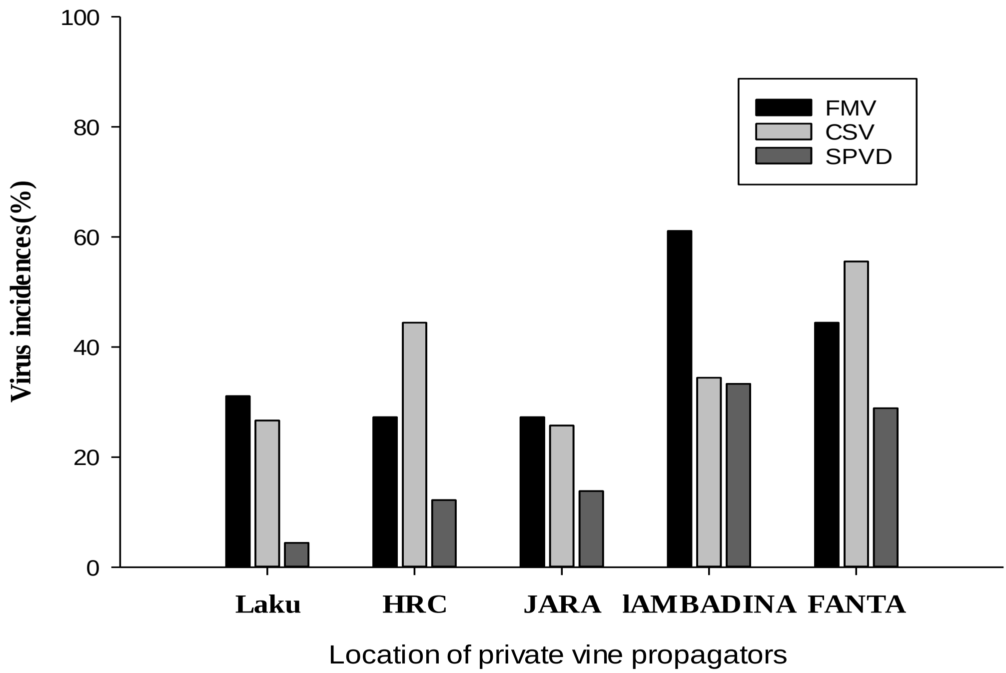

Figure 4. Incidences of viruses in commercial vine propagators' fields in Southern Ethiopia; the incidence was calculated for each virus regardless of varieties tested.

Table 3. Incidences of SPFMV, SPCSV and mixed infections in samples collected from two varieties of sweet potato planted at farmers' field, vine propagators' and Hawassa Research Center' collections.

Propagators/ multipliers	Variety	Incidences of viruses (%)		
		SPFMV	SPCSV	SPFMV + SPCSV (SPVD)
Hawassa Research Center	Hawassa-83 & other breeding line	27.3	44.44	11.7
	Kulfo	40	46.7	13.1
Bilate (Jara)	Hawassa 83	1.5	30.8	0
	Kulfo	53.9	21.5	13.8
Lambadina	Hawassa 83	44.4	6.7	6.7
	Kulfo	77.8	62.2	53.3
Leku	Hawassa 83	28.3	18.3	1.7
	Kulfo	36.4	43.3	10.0
Sodo zuria (Fanta farm)	Hawassa 83	44.4	55.6	28.9
Farmers' fields	Hawassa 83	28	47.1	19.5

The sweet potato field observations in study areas in Southern Ethiopia reveal existences of plants having severe and mild virus-like symptoms. In addition, ELISA test results evidenced high infection level by the two common viruses, SPFMV and SPCSV. When there exist high infection level and planting materials is freely exchanged between farmers and location, virus/es dissemination occurs mostly through infected planting material. The high level detection of SPFMV and SPCSV as a single and mixed infections, given the poor regional and national quarantine systems in the country makes challenging of getting virus-free planting and critical to sweet potato production and future dissemination in the country. The yield reduction impact SPFMV is low specially when it infect resistant cultivars that have an inherent ability to become virus-free (Karyeija *et al.*, 1998). On the other hand, farmers have little to moderate knowledge of viruses that are infecting their sweet potato plant and received very little or no trainings in virus protection (Dereje Haile, 2019).

Conclusion and Recommendation

The present virus survey showed commercial vine multiplication fields, research center and private farmers' sweet potato crops in the fields are infected with one or two of the virus/es tested. Compared to the previous studies, the incidences of each virus and their co-infections are increasing in the surveyed areas. Survey revealed high incidences of SPFMV, SPCSV and their mixed infection commonly causes SPVD, indicating the less viability of the current exiting functional clean seed system. As planting materials has been disseminated to many parts of the country, there is need for future study to generate national and regional disease prevalence map to identify areas with a high disease incidence and severity.

As stated before, previous studies and this study depicts the necessity of generating clean planting materials, providing disease free basic seeds to vine propagators, strong follow-up and quarantine system to solve the problem related to virus infection in sweet potato fields. Furthermore, this study underscores the need to train farmers on virus management practices such selecting healthy planting materials, early identification of symptoms and rouging out of infected plants to limit the distribution of infected planting materials to were no reports of these viruses.

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