Pest Management Journal of Ethiopia

Volume 16, 2012
ISSN 1028 – 0308

Plant Protection Society of Ethiopia

Introduction

Potato (*Solanum tuberosum* L.) was introduced to Ethiopia by the German botanist, Schimper, in 1858. Since then it has become a staple food crop in many parts of the country though its acceptance was very slow that took several decades (Pankhurst, 1964). It is grown by about 1 million farmers mainly during the rainy season in agro-ecologies with annual rainfall above 600 mm at altitudes ranging from 1,500 - 3,000 m above sea level (Central Statistics Agency (CSA), 2008/2009). Potato is usually grown as a mono crop and seldom rotated with cereal or leguminous crops (Adane *et al.*, 2010).

Recently, it is widely produced in the central, southern, northwestern and eastern highlands of Ethiopia that accounts about 83 % of the potato farmers in the country (CSA, 2008/2009). The total areas under potato production are estimated to be 160,000 ha. The national average production is about 8 tons / ha, which is less than the average yield of the African continent (10.8 tons / ha) (Food and Agricultural Organization Statistical database (FAOSTAT), 2008).

In potato production, quality of seed potato is an important determining factor in the quality and quantity of the final product. Among various biological factors limiting potato production in Ethiopia viral, fungal and bacterial wilt diseases appear to be significant production constraints. This is mainly due to lack of healthy seed potato suppliers in the country (Bekele *et al.*, 2011 and Adane *et al.*, 2010).

Now-a-days, over 30 viruses and strains are known to infect potato and cause significant damage in different potato growing parts of the world (Salazar and Accatino, 1990). The most economically important viruses are found in genera Luteovirus, Potyvirus, Potexvirus and Carlavirus (Valkonen, 2007). Studies conducted in mid 1980's in central, south and Southeast Ethiopia (Agranovsky and Bedasso, 1985) and recently in Western Amhara (Bekele *et al.*, 2011) showed the presence of PVY, PVX, PVA PVM, PVS and PLRV. Potato virus Y (PVY) is a serious challenge for seed potato growers in developing countries particularly in a mixed cropping system causing 50-80 % yield losses in all potato growing regions in the world (Miha *et al.*, 1993; Singh and Somerville, 1992 and Valkonen, 2007). It can be controlled by genetic resistance and seed certification schemes in long and short terms respectively (Hiskias *et al.*, 1999 and Singh and Somerville, 1992). PVS causes asymptomatic infections in most potato varieties that result in up to 20 % yield losses (Valkonen, 2007). It also causes mixed infections with potato virus M, X or some other potato viruses causing more severe damages. PLRV is one of the most damaging and widely spreading potato viruses and found wherever the potato crop is grown. It has been reported to cause tuber yield losses ranging from 33-55%. Other potato viruses namely PVA, PVX and PVM are also economically important potato viruses worldwide that cause potato tuber yield losses of 30 %, 5-15 %, and 10 % respectively (Miha *et al.*, 1993 and Muthomi *et al.*, 2011).

In the process of infection, viruses usually damage or disturb a plant's normal growing pattern, resulting in visible symptoms such as leaf distortion, leaf mottle, chlorotic appearance, leaf mosaic and plant stunting. Furthermore, virus infections also cause retarded plant growth, reduced photosynthesis, inefficient use of nutrients and increase of plant vulnerability to other stresses (CIP, 2007).

Currently, increase in potato farms and potato demands in Ethiopia require quality improvement of the seed potato and ware potato production systems that need better information about biotic and abiotic stresses which affect potato yields. Potato viral diseases are one of the common biotic factors that have been known to influence potato productions in this country.

However, information on the economically important major potato viruses is insufficient in Ethiopia though very few reports have shown the incidence in a few hot spot locations in the country (Bekele *et al.*, 2011and Adane *et al.*, 2010). To suggest options for a proper management and to design appropriate control measures, knowledge of the pathogen existence, distribution and their occurrences are the key elements that need to be addressed appropriately. Thus, this study was aimed at assessing the incidence and current status of major potato

Incidence and Distribution of Potato Viruse Diseases in West Arsi and West Shewa Major Potato Growing Areas, Ethiopia

[1]Seifu Juneidi, [1]Tariku Megeressa, [2]Berhanu Bekele and [3]Belayneh Admasu
[1]Ambo University, P.O.Box 19, Ambo, Ethiopia
[2]Ambo Plant Protection Research Center, E-mail: bekeleberhanu56@yahoo.com
[3]Holetta Agricultural Research Center, Holotta, Ethiopia

Abstract

Field surveys were carried out in West Arsi and West Shewa administrative zones to determine incidence of potato viruse diseases. Limited information is available on major potato viral diseases in these areas. Virus-like symptomatic leaf samples were collected from 13 districts in 71 fields for the serological detection of the common potato viruses. Field disease incidence was visually assessed based on viral disease symptoms and the identities of the viruses were tested by double antibody sandwich (DAS)-ELISA. During the field surveys, virus-like symptoms were more common in West Arsi than in West Shewa. The serological test results showed that potato virus X (PVX) (50 %) was the most predominant followed by potato virus S (PVS) (38 %), potato leaf roll virus (PLRV) (11 %) and potato virus Y (PVY) (2 %). Mixed infections of PVX and PVS were noticed in all surveyed areas, while PVY co-infection was the least detected combination with any of the tested viruses.The high incidence of PVX and PVS is most likely attributed to their mode of mechanical transmissions. PLRV transmission by aphid in a persistent manner might have favored its higher incidence as compared to PVY that is transmitted by aphids in a non-persistent manner. Of the total assayed samples, 93 %, 86 %, 73 %, 74 %, 71 %, 70 %, 69 %, 59 %, 57 %, 50 %, 48 %, 20 % and 19 % at Arsi-Negele, Shashamne, Kofele, Ambo, Jibat, Holeta, Adea-Berga, Guder, Tikur-Inchini, Wonchi, Jeldu, Dendi and Elifeta were tested positive for at least one of the assayed viruses, respectively. The high occurrence of the viruses could be attributed to different factors including recycling of the infected potato tubers and cropping system, overlapping of potato growing seasons, presence of biological vector inside potato field and infected volunteer potato from previous seasons. Overall, more than 50 % of the assayed samples were tested positive for at least one virus in the majority of the surveyed fields. Current crop yield losses due to the pathogen need to be quantified with independent studies so as to determine the magnitude of the problem in these areas.

Key words: DAS-ELISA, Mixed infection, Potato virus, Single infection, Survey

viruses in main potato growing areas in West Shewa and West Arsi administrative zones, Ethiopia.

Materials and Methods

Study areas

The surveys were conducted in two important potato growing administrative zones (West Arsi and West Shewa) in Oromia regional state, Ethiopia (Fig. 1). The altitudes of surveyed areas ranged from 1900 masl at Shashamene to 2600 masl at Ars-Negele in west Arsi, and 1933 masl at Senkele to 3032 masl at Dandi in west Shewa zone. The geographical positions of the study areas were between N7°4'– N7°41 and E38°47 – E39°15' in west Arsi, and between N8°17'– N9°60' and E37°17 – E38°45'in west Shewa. The size of inspected potato fields were between 0.25 to 3 ha in farmers fields and 3m x 5m =15m^2 at experimental plots.

Sample collection

The sample domains were selected to represent the major potato producing zones from warmer areas in the Ethiopian Rift Valley and relatively cooler highland areas of the country. Major potato growing locations were selected by consulting agricultural offices of the respective zones.

The samples were collected during a short rainy season (January – June, 2012) mainly in irrigated potato farms. The average distance between two nearby randomly sampled fields was 5 km, and 5 - 12 plant samples per field were collected during the field visits. A total of 608 leaf samples were collected from 67 fields for laboratory analysis. The leaves were collected from lower, middle and upper parts of a plant and each leaf sample was placed in a separate plastic bag and immediately transported in ice box to Ambo plant protection research center. Potato leaf samples were collected at flowering and tuber setting stages (about 9 - 11 weeks). Symptomatic and non-symptomatic samples were collected from seed potato farms, ware potato farms and experimental plots. Emphasis were given to symptomatic ones (Table 1). Sampling of the field was performed with a simple random sampling technique followed by diagonal sample collections in a sampling area of 20 m^2 (4 m x 5 m). The number of samples collected from each field ranged from five to twelve depending on the diversity and distribution of the symptoms encountered in the respective potato fields.

During potato field inspections, information on source of seed potato tubers, growth stage and potato cultivars, disease symptoms, field disease incidences, disease management practices, purpose of production (ware, seed or research), altitude, geographical position, field size and location of the study areas were recorded.

Serological Assays: DAS-ELISA

The serological assays were done using the Double Antibody Sandwich-Enzyme Linked ImmunoSorbent Assays (DAS-ELISA) method as described by Clark and Adams (1977). The tests were done for the presence of six economically important potato viruses namely PVX, PVY, PVA, PVM, PLRV and PVS with ELISA kits obtained from the international potato center (CIP). All samples were tested in duplicates according to a standard protocol described in CIP DAS-ELISA kit. Briefly, composite of bottom, middle and top leaf samples of a plant (1 g) were extracted in 4 ml of the extraction buffer [4 g polyvinylpyrrolidone (PVP) - 40000 and 2 g egg albumin dissolved in 200 ml of PBS buffer]. Extraction was done using a pestle by pressing on a flat surface of a plastic bag containing leaf samples. The polystyrene microtitre plate was coated with 100 µl of coating solution containing 0.2 g Na_2CO_3, 0.44 g $NaHCO_3$, 0.03 g NaN_3, 30 ml distilled water and 35 µl of antibody (IgG) of the virus to be detected. The plates were sealed with parafilm and incubated at 37 ^{0}C for 4 hr. Emptied and tissue paper dried plates were then washed three times with phosphate buffered saline-tween (PBS-T) (8 g NaCl, 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, 0.2 g KCl, 0.195 g NaN_3, 1000 ml distilled water and 0.5 ml tween 20) at 3 min intervals. Each well was loaded with 100 µl of antigen (buffer extracted leaves saps), sealed with parafilm and incubated in refrigerator at 4 ^{0}C overnight. Extracts from healthy plants and infected plants were used as negative and

positive controls, respectively. The wells were washed three times with PBS-T and filled with 90 µl conjugate solution (35 µl of respective virus antiserum conjugated with alkaline phosphatase (IgG-AP), 0.4 g PVP-40000, and 0.04 g egg albumin dissolved in 20 ml PBS-T) and incubated at room temperature for 4 hr. The wells were then washed three times with PBS-T and loaded with 80 µl substrate solution (17.46 ml Diethanolamine, 9.6 ml distilled water, 2.4 ml HCl (37 %) and 0.5 mg/ml of *p*-nitro phenyl phosphate) and kept at room temperature for 1 hr for the reaction to take place. A positive reaction was revealed by the development of yellow color and its absorbance was determined by a spectrophotometer at 405 nm (Anthos Labtec HT2, Version 1.20E) according to $x \geq \bar{x}\, h \times 2$ relationship, where x = positive sample, $\bar{x}$ h = average value of healthy controls and 2 = standard deviation. To estimate relative plant virus titers, positive samples were compared based on absorbance values at 405 nm in a single microtiter plate (Singh and Somerville, 1992).

Fig 1. Map of Ethiopia showing study areas in central Ethiopia.

Data Analysis

Field disease incidence was stratified based on symptomatic plant samples and incidence of viral infections were expressed as the percentage of virus infected plant samples (James, 1974). The obtained data were analyzed using Statistical Package for Social Science (SPSS) version 17 software to determine means and percentages. Least significant differences (LSD) of mean separations at 5% confidence interval and correlation coefficients were analyzed by student's t-test.

RESULTS

Field diagnosis

The common virus-like symptoms observed during sample collection include leaf mottling, leaf mosaic, chlorotic spots, leaf necrosis, leaf curling, plant stunting, leaf distortion, reduced leaf sizes and deepening of leaf veins. Chlorosis, leaf curling and leaf mosaic were the most commonly noticed symptoms in the potato fields. In addition, plants showing purple pigmentation at the base and tip of leaflets, and pallor leaf symptoms were also noticed in some fields (Fig. 2 A-F). Virus-like symptoms were

observed in the majority of the inspected potato fields, in both administrative zones. Field disease incidences varied between the two zones and among fields within a zone. It was generally higher in West Arsi zone than West Shewa zone.

Based on field observation, average disease incidence in the surveyed fields ranged from 9 % to 55 % depending on the potato sampling sites, potato varieties and purpose of productions. The highest field disease incidence was recorded at Shashamane (55 %) followed by Kofele (52 %), Arsi-Negele (47 %), and Guder (41 %), and the least at Tikur-Enchini (9 %) seed potato producer's cooperatives field. Overall, the disease incidences were low in seed potato farms (farmer's seed potato field and seed potato producer cooperatives) compared to ware potato farms and variety trial experimental plots.

In many of the sampled areas (4 m x 5 m), within a surveyed field, field disease incidences were recorded between 26-35 % except at Tikur-Inchini, Wonchi and Holetta where the disease occurrences were less than 5 %, between 5-15 % and between 16-25 % respectively. At Tikur-Inchini two of the sampled areas were non-symptomatic to virus-like diseases, and 56-65 % virus-like symptoms were recorded at Guder, Ambo, Dendi and Adea-Berga sampled areas. One third of the sampled areas at Kofele, Shashamene and Arsi-Negele had > 65 % incidence of virus-like symptoms. Four of eight sampled fields at Guder (four experimental plots) showed 100 % virus-like symptoms. (Table 1).

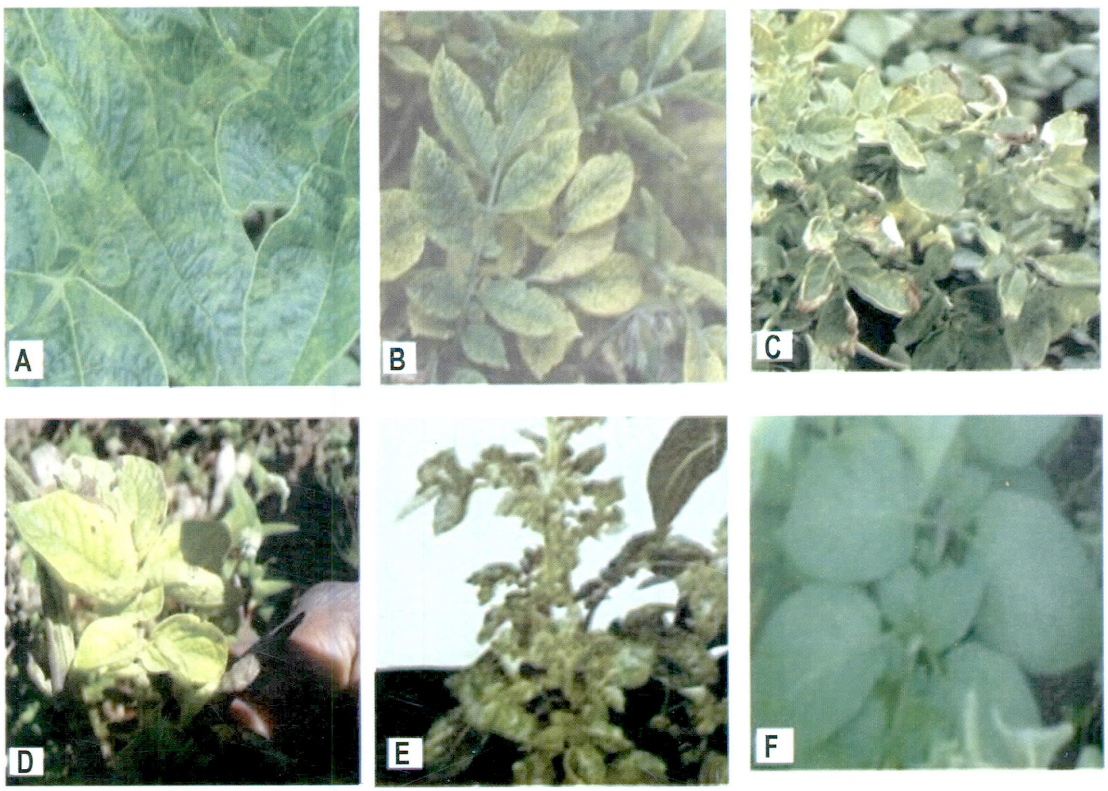


Fig. 2. Some virus-like symptoms observed during the field surveys. A, leaves curling, curve inward and stiff upward growth; B, leaves mosaic; C, leaves mottling and necrosis; D, leaves chlorosis; E, leaf deformation and dwarfing and F, Asymptomatic healthy looking plant

Table 1. Virus disease incidence based on observed symptoms in farmers' potato fields and in research experimental plots in West Shewa and West Arsi administrative zones during the short rainy season, 2012.

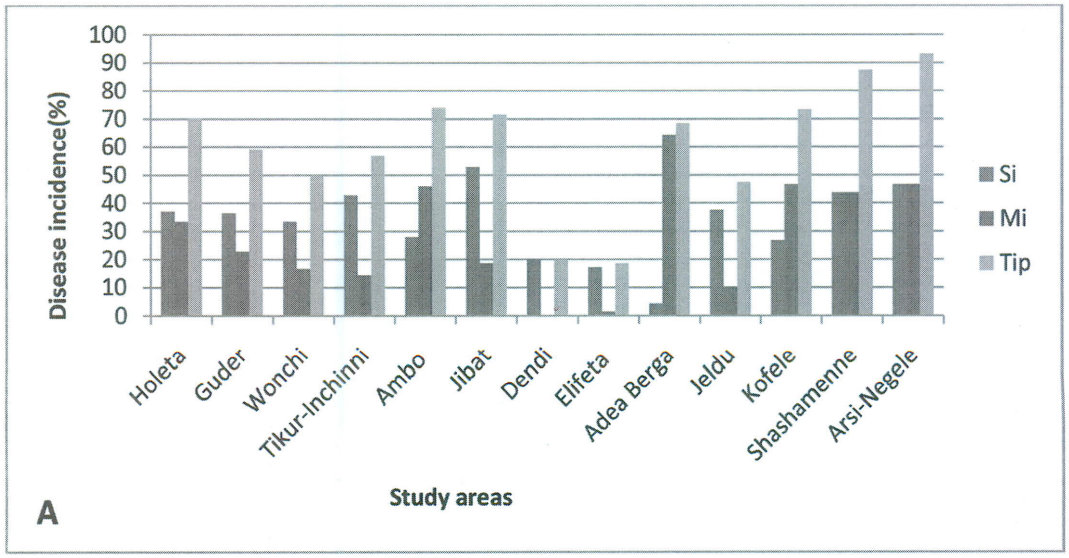
Zone	Surveyed Areas	Farm type	No. of fields	No. of samples	No. of the sampled area with disease incidence (%) of:								
					<5	5-15	16-25	26-35	36-45	46-55	56-65	>65	AFDI (%)
West Shewa	Guder	[a]EP/FF	8	66	1	11	12	21	5	6	5	1+4**	41
	Holetta	FF	5	30	9	5	10	3	3				18
	Wonchi	FSPE	2	12	4	5	3						12
	Tikur-Inchini	SPPC	2	14	5+2*	5	2						9
	Ambo	FF/FSPF	5	50	7	10	9	15	2	4	3		31
	Jibat	FF/FSPF	5	70	9	10	23	27	1				24
	Dendi	FF/FSPF	14	140	16	42	23	49	9		1		17
	Elifeta	FF/FSPF	7	70	8	16	19	25	2				21
	Adea-Berga	FF	7	70	7	13	21	22	2	3	2		38
	Jeldu	FF/FSPF	4	40	7	5	9	14	3	2			19
West Arsi	Shashmene	FF/FSPF	4	16		1	2	5		2		6	55
	Kofele	FF	4	15	1	4	2	1	1			6	47
Arsi-Negele	FF		4	15	1	4	5					5	52

EP, Experimental Plot; FF, Farmers' ware potato field; FSPF, Farmers seed potato field; SPPC, Seed Potato Producers Cooperatives; AFDI, Average field disease incidence; Sampled area, 4 m x 5 m quadrant; *,Virus-like symptoms free sampled areas; **, 100 % virus-like symptomatic experimental plot; a, Five experimental plots sampled at Guder research station. The numbers of the sampled areas were equal to the total number of samples collected from each surveyed fields. That is, from each quadrant one sample was collected for serological test. Each of the experimental plots was considered as a field and field disease incidence was recorded per plot. Field disease incidence (%) = $\frac{\text{No. of symptomatic samples}}{\text{Total plants}} \times 100$

Incidence of Potato Infecting Viruses

Six major potato viruses namely PLRV, PVX, PVS, PVY, PVM and PVA were serologically assayed to assess their incidences in the surveyed areas. The detected viruses and their incidences are shown in fig. 3. PLRV, PVX and PVS were detected with significant differences ($p \leq 0.05$) in many areas of the surveyed fields. The virus disease incidences were generally higher in fields assessed in West Arsi than in West Shewa. Forty eight percent and 85% of the samples collected from West Shewa and West Arsi were tested positive for at least one virus, respectively. The highest virus infected samples were obtained from Arsi-Negele (93 %), followed by Shashamene (88 %), Ambo (74 %), Kofele (73 %) and Jibat (71 %) areas studied, and the disease incidences were about the same at Adea-Berga and Jibat areas surveyed. Relatively, the viral disease occurrence was lower at Elifeta (19 %), Dendi (20 %), Jeldu (48 %), Wonchi (50 %) and Tikur-Inchini (57%) compared to other fields surveyed.

Infection of PVX occurred most commonly in all areas except at Shashamene and Jeldu where PVS was the most frequent. The incidence of PVS was higher than PVY, PVM, PVA and PLRV in all surveyed areas apart from Kofele and Arsi-Negele where it had similar incidence with PLRV. The occurrence of PLRV ranged from 0 % to 40 %. PVY was detected only in samples collected from Guder and Kofele and it was the least common compared to other viruses detected. PVM was detected in only 4.3 % of the samples collected from Adea-Berga, and PVA was not identified in any of the samples collected from all the surveyed fields. Moreover, 30 % of the symptomatic samples, which were assumed to be viral diseases, reacted negatively to anti-sera used in this study. Overall, PVX was the most frequently detected virus (50 %) followed by PVS (38 %) and PLRV (11 %) (Fig.3B)



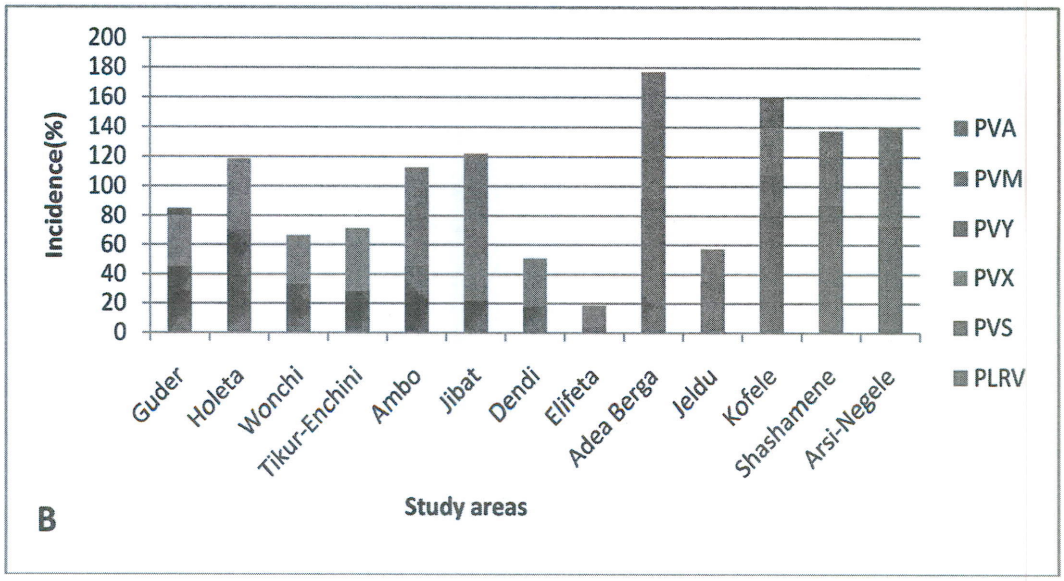


Fig. 3. Incidence of potato viruses in samples collected from farmer's potato field during a short rainy season. A, Percentage of single, mixed, and total virus infections; B, Percentage of individual virus infection; PLRV, Potato leaf roll virus; PVX, Potato virus X; PVY, Potato virus Y; PVS, Potato virus S; PVA, potato virus A and PVM, potato virus M.

Single and mixed viral infections

The assay results were also assessed for single and mixed infections of the detected viruses (Fig. 3A). On average, 33 % and 28 % of the samples had single and mixed infections respectively. At Holeta, Guder, Wonchi, Tikur-Inchini, Jibat, Elifeta and Jeldu majority of the potato samples had a single virus than multiple virus infections. On the other hand, mixed infections were more common compared to single infections at Kofele, Ambo and Adea-Berga areas. Single and mixed infections were the same in samples collected from Shashamene and Arsi-Negele areas.

Except Dendi, multiple infections involving two or more viruses were found in all the surveyed fields (Table 2). Mixed infections of three viruses were detected in samples collected from Holeta, Guder, Adea-Berga, Kofele and Shashamene, and one sample, from Kofele, was tested positive for four multiple virus infections. Apart from PVA and PVM, all mixed infection types were detected in Kofele areas. The most commonly obtained virus combinations were PVX and PVS. It was the highest at Adea-Berga (63 %), followed by Kofele (45 %), Shashamene (44 %) and Arsi-Negele (40 %) areas, while the least combination of these viruses was recorded in fields sampled at Wonchi (8 %) areas. Mixed infections of PLRV with either PVS or PVX were more common compared to the remaining virus co-infections. PVY multiple infections, with any of the assayed viruses, were the least frequently detected. Combination of PVA and PVM with any of the assayed viruses were not tested positive for any mixed infection types.

Table 2. Disease incidence of mixed infections of PVX, PVS, PVY & PLRV in the surveyed potato fields

Study areas	% incidence of each mixed infection type									
	PLRV +PVS	PLRV+P VX	PLRV+P VY	PVS +PVX	PVS +PVY	PVX +PVY	PLRV +PVS +PVX	PLRV+P VS+PVY	PVS +PVX +PVY	PLRV +PVS +PVX +PVY
Holeta	11	15	0	30	0	0	11.1	0	0	0
Guder	9.1	4.5	0	9.1	3	1.5	1.52	0	1.52	0
Wonchi	0.0	8.3	0	8.3	0	0	0	0	0	0
Tikur-Inchini	0.0	0	0	14	0	0	0	0	0	0
Ambo	0.0	0	0	46	0	0	0	0	0	0
Jibat	0.0	0	0	21	0	0	0	0	0	0
Dendi	0.0	0	0	0	0	0	0	0	0	0
Elifeta	0.0	0	0	1.3	0	0	0	0	0	0
Adea-Berga	15.7	15.7	0	63	0	0	15.7	0	0	0
Jeldu	2.5	2.5	0	5	0	0	0	0	0	0
Kofele	20	33	20	45	6.7	20	20	7	6.67	6.7
Shashamene	6.3	6.3	0	44	0	0	6.25	0	0	0
Arsi-Negele	6.7	0	0	40	0	0	0	0	0	0

Plus (+) sign indicates mixed infections

Incidence of each mixed infection type (%) = $\frac{\text{No of positive samples for mixed infections}}{\text{Total assayed samples}} \times 100$

Concentration of the detected viruses was measured so as to estimate the amount of each virus in the tested samples. On average, the highest mean virus titer was measured for PVS, followed by PVX, and PLRV, and the lowest for PVM (Table 3). There were significant ($p \leq 0.05$) differences in virus titer among the inspected areas except, for PVY, PVA and PVM. The highest virus titer for both PLRV and PVS were detected in samples collected from Kofele, and the least in samples obtained from Wonchi and Holeta, respectively. The highest PVX titer was recorded in samples collected from Guder and the lowest in samples from Jeldu areas. PVY had a mild ELISA reaction in samples obtained from Guder and Kofele areas.

Discussion

As potato productions is highly increasing in Ethiopia, problems associated with viral diseases need to be addressed properly. Similar to our field survey result, it was reported that the most commonly observed virus infected plant symptoms were mottling, mosaic, crinkle, chlorotic spots, necrosis, leaf curling, stunting, leaf distortion, reduced leaf sizes and deepening of leaf veins (Bekele *et al.,* 2011; Pourrahim, 2007 and Miha *et al.,* 1993). Thus, the results obtained from our current field observations and laboratory tests showed that virus diseases could be among limiting factors of seed and ware potato productions in Ethiopia. Virus diseases are one of the main constraints to produce potato worldwide as there are no efficient chemical treatments (Biniam and Tadesse, 2008 and Ullman *et al.*, 1991). In some fields (such as in Shashemene, Arsi-Negele and some experimental plots), improved potato varieties were more susceptible to viral diseases compared to local potato cultivars. This can be explained by the fact that continuous breeding to increase yield might have resulted in loss of pathogen resistant traits and /or the local potato cultivars adapted to the pathogens over time. Plant breeding, with many advanced agricultural technologies, has made remarkable advancement in increasing crop yields for over a century. However, continuous inbreeding and selective breeding of particular genes has the risk of losing some of the other important genes from the gene pool altogether that could increase susceptibility of a plant to specific pathogens (Evans, 1997).

The survey result showed that four of six assayed viruses (Viz., PLRV, PVS, PVX and PVY) were present in many of the surveyed areas. This is a reason for concern since these viruses are known to be economically important in developing countries (Salazar and Accatino, 1990). The viral diseases were common in both surveyed administrative zones though there were considerable differences in incidence among the surveyed fields (Fig. 3). The highest disease incidence was recorded in samples collected from West Arsi compared to West Shewa. At Arsi-Negele, Shashamenne and Kofele (West Arsi zone) the disease incidence was 93 %, 88 %, and 73 % respectively, whereas in West Shewa the highest and the least virus disease occurrences were documented at Holeta (70 %) and Wonchi (10 %) in the same order. This difference is partly explained by sources of seed potato tubers. Except in one field at Shashamene (farmer's seed potato field), farmers in West Arsi zone obtain the potato seed tubers from the local open market and/or make use of their own saving from previous seasons of production. On the other hand, the sources of potato seed tubers for Wonchi, Tikur-Inchini, Guder and Holeta were the nearby Holeta Agricultural research center. At Holeta, one of the five potato fields was planted with seed tubers from Shashamene area local market that showed higher incidence for assayed viruses. Higher occurrence of viruses in this field supports the previous explanation for the differences in viral disease incidence in the surveyed fields in the two administrative zones.

Three of the six viruses, namely PVS, PVX and PLRV, were widely distributed in all surveyed areas. Thus, high incidence of these viruses in ware potato and seed potato production is of great concern. PVX, PVS and PLRV caused 5-15 %, 10-20 % and 50-90 % yield losses respectively depending on potato cultivars, virus strains and climate (Jones *et al.* 2009). PVX and PVS were the most common and widely distributed in the surveyed fields which was consistent with similar survey report from

Amhara regional state, Ethiopia (Bekele *et al.* 2011), Iran (Pourrahim *et al.*, 2007) and Nigeria (Miha *et al.* 1993). According to Jones *et al.* (2009), these viruses occur most frequently worldwide and are known to cause mild mosaic symptoms. Comparatively, PLRV was more common than PVY, PVM and PVA in all the fields surveyed, and this finding agrees with surveys conducted in Kenya and Belarus (Muthomi *et al.*, 2011 and Bloskaya, 2000). PVY and PVM were rarely detected in samples from most surveyed fields, and PVA was not detected in any field samples. This finding was comparable with a similar study conducted in northern Ethiopia (Bekele *et al.*, 2011), and contradicts with other studies conducted in Iran (Pourrahim *et. al.*, 2007), USA (Baldauf *et al.*, 2006) and Lebanon (Yusuf *et al.* 2001) that reported PLRV, PVS, PVX , PVY, PVM and PVA are the major potato viruses. The most likely reason for the high incidence of PVS and PVX is their transmission by mechanical contaminations. PVS and PVX are mainly transmitted mechanically through equipment, contact and/or infected tubers (Muthomi *et al.*, 2011 and Solomon-Blackburn and Barker, 2001). During farm operations PVS and PVX easily spread that accounts for their higher incidence compared to PLRV, PVY, PVM and PVA (Miha *et al.*, 1993). Hooker (1982) reported that humans transmit potato viruses by mechanical contact causing leaf or sprout contact from infected to healthy plant while planting, weeding, sorting, and/or harvesting procedures. According to the same authors some viruses survive for some time on clothes, hands, hoes or cultivar that disseminate during field operations.

On the other hand, persistent transmission of PLRV by aphid might have favored its higher incidence compared to PVY, PVM and PVA that are transmitted by aphids in a non-persistent manner. PLRV is transmitted in a persistent manner by the green peach aphid, *Myzus persicae* whereas PVY is vectored by *Myzus persicae, Aphis fabae, Macrosiphum euphorbiae* and *Rhopalosiphum insertum* in a non-persistent manner (Muthomi *et al.,* 2011 and Solomon-Blackburn and Barker, 2001). According to Edwards (1993), PVY is less efficiently transmitted by several non-colonizing vectors, with the most important being the bird cherry-oat aphid, *Rhopalosiphum padi* L. Srinivasan and Alvarez (2007) also reported that persistently transmitted viruses (example, PLRV) attract and arrest their vectors by virus induced volatiles to encourage sustained feeding compared to non-persistently transmitted viruses, such as PVY and PVA, that support shorter feeding periods. According to this preliminary survey, PVY, PVM and PVA are of minor economic importance in both West Arsi and West Shewa administrative zones, though occurrences and distributions of these viruses require further biological and molecular investigations.

Several factors may have contributed to higher occurrences of these potato viruses in the surveyed areas: recycling of old infected tubers, overlapping potato growing seasons, infected volunteer plants (arising from tuber from previous crop), potato cultivars, cropping systems, mechanical contacts, biological vectors and vegetative propagations. The most prominent among these could be the continuous recycling of old infected seed tubers without replacement. In Ethiopia, in all potato growing areas, the majority of farmers use seed potato tubers of unknown origin from the local open market or from their own saving from previous season of productions (Adane *et al.*, 2010). In the absence of well established healthy seed multiplication and distribution systems, farmers resort to choose seeds from the previous season of ware potato productions or use leftover tubers from local open market (Biniam and Tadesse, 2008). According to these authors such seed tubers are small tubers with little or no market values. Furthermore, the high incidence of these viruses also partly explained by reservoir host plants that grew inside and/or outside potato fields. Almost in all surveyed areas, tomato and pepper fields were found near potato fields, and even intercropped with tomato in some fields. Some weeds (Amaranthus *spp.*, Chenopodium *spp.*, *Datura stramonium*, *Solanum incanum* and Nicandra *spp.*) were also widely distributed inside and/or outside potato fields. Major potato viruses were previously reported from these plants as alternative hosts (Hiskias *et al.*, 1999 and Kook-Hyung, 2001). However, independent studies of biological vectors (aphids and other insects), environmental factors, origin of potato seed tubers and potato cultivar, alternative host

plants and viral strains should be done to identify the main causes for high incidence and distribution of potato viruses in these areas.

In parts of the world where advanced science and technology of seed potato programs are in operation or high quality seed potatoes are imported, mixed infections of potato with more than one virus is now less frequent than in the past. But, it is still common in areas where less quality seed potato tubers are grown (Jones *et al.*, 2009). On average, 28 % and 33 % of the total tested samples were infected with mixed and single viruses, respectively. Mixed infections with two or more viruses were frequently detected in all surveyed fields, of which PVS and PVX combinations were the most common (Table 2). Co-occurrences of PVY with any of the commonly detected viruses (PLRV, PVS or PVX) were significantly less than co-infections of PLRV, PVS or PVX. This finding agrees with the survey conducted in central, south and southeast Ethiopia (Agranovsky and Bedasso, 1986) and in northern Ethiopia (Bekele *et al.*, 2011). It was inconsistent with a similar survey carried out in Nigeria (Miha *et al.*, 1993) in which PVX and PVY combination was the most common. Srinivasan and Alvarez (2007) reported frequent co-infections of PVY with PLRV in USA that also contradicts with our result. Thus, the incidence of PVY is less or the anti-serum used for the detection might not be specific to the PVY strain(s) in Ethiopia or even there might be uncharacterized PVY strain(s) exist (s) in Ethiopia. Furthermore, 33 % of the samples displaying virus-like symptoms were sero-negative for assayed viruses suggesting the possible presence of other viruses, or other unidentified causative agents. Bekele *et al.* (2011), Pourrahim *et. al.* (2007 and Hiskias *et al.* (1999) also noticed similar results in their sero-diagnosis based survey of potato viruses.

Virus titers of the assayed leaf samples were also measured (Table 3) to determine relative concentrations of each virus in infected plants although several factors viz. prevalence of biological vectors, time between inoculation and assay, type of virus, interaction of viruses (in case of mixed infections) and susceptibility of potato cultivar...are likely to affect virus concentration under field conditions. Except for PVY and PVM, virus titers of PLRV, PVS and PVX were insignificantly different ($p \geq 0.05$) in the surveyed fields. Thus, the high virus titer of PVS, PLRV and PVX reflects a very high inoculum reservoir (for example, infected tubers) and/or high prevalence and efficient aphid vectors in transmitting potato leaf roll viruses (PLRV) in the surveyed fields. This result agrees with the study conducted by Muthomi *et al.* (2011) in Kenya on seed potato tubers from retail markets and farmers' potato stores.

Table 3. Mean virus titer in potato leaf samples collected from West Arsi and West Shewa zones.

Study areas	PLRV	PVX	PVY	PVS	PVM
Guder	0.300	0.549	0.222	0.50	ND
Holeta	0.217	0.345	ND	0.14	ND
Wonchi	0.141	0.207	ND	0.19	ND
Tikur-Inchini	0.180	0.262	ND	0.27	ND
Ambo	ND	0.232	ND	0.26	ND
Jibat	ND	0.290	ND	0.37	ND
Dendi	ND	0.341	ND	0.35	ND
Elifeta	ND	0.410	ND	0.42	ND
Adea-Berga	0.27	0.250	ND	0.27	0.26
Jeldu	0.20	0.213	ND	0.22	ND
Kofele	0.478	0.291	0.255	0.57	ND
Shashamene	0.416	0.206	ND	0.35	ND
Arsi-Negele	0.470	0.205	ND	0.47	ND

Values are mean absorbance measurement at 405 nm from ELISA tests. Larger values indicate higher virus titer. ND, Not detected; PLRV, Potato leaf roll virus; PVX, Potato virus X; PVY, Potato virus Y and PVS, Potato virus S.

Subsequently, in addition to generating baseline information on major economically important potato viruses and their geographic distribution, it is of practical significance to inform and make farmers aware and practice on a range of available disease management alternatives, with due emphasis on affordable disease management options. It is also hereby suggested that the occurrence of potato viral disease in a field should be considered as one of the major criteria in the national potato improvement program to avoid the release and distribution of susceptible varieties to farmers. Planting healthy potato seed tubers (that are certified by a recognized seed potato inspection program) and internal quarantine are recommended to be implemented to effectively minimize the spread of the pathogens. Furthermore, the crop losses due to potato viruses should be quantified across the surveyed areas with independent studies so as to determine the magnitude of the problem.

Acknowledgement

The authors would like to extend heartily gratitude to the National Biotechnolony Laboratory, Holetta Agri. Research centre, EIAR for providing DAS-ELISA kits. Deep pleasure and gratefulness also extended to the Ambo Plant Proection Research Centre (PPRC) for providing research facilities and space in virology laboratory to conduct serological studies.

References

Adane H., Miranda P. M., Agajie, T., Willemien, J. M., Alfons, O. L., Admasu, T. and Paul C. (2010). Analysis of Seed Potato Systems in Ethiopia. Am. J. Pot Res.

Agranovsky A.A., Bedasso J. (1986). Survey on virus diseases of potato and tomato in major growing areas. Scientific Phytopathological Laboratory (SPL) Progress Report for 1985/86:247-249.

Baldauf P.M., Gray S.M. and Perry K.L. (2006). Biological and Serological Properties of Potato virus Y Isolates in Northeastern United States Potato. Plant disease 90:559-566.

Bekele B., Abate E., Asefa A. and Dickinson M. (2011). incidence of potato viruses and bacterial wilt disease in the west amhara sub-region of ethiopia. Journal of Plant Pathology 93: 149-157.

Biniam T. and Tadesse M. (2008). A survey of viral status on potatoes grown in Eritrea and in vitro virus elimination of a local variety 'Tsaeda embaba'. African Journal of Biotechnology 7:397-403.

Blotskaya V.Z. (2000). Economic importance of potato virus disease distribution in the Republic of Belarus, Archives of Phytopathology and Plant Protection, 33:1, 81-86

CIP (2007). Procedures for standard evaluation trials of advanced potato clones. An International Cooperators' Guide.

Clark M.F. and Adams A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. *Journal of General Virology* 34:475-483.

CSA (2008/2009). Agricultural sample survey: Report on area and production of crops, Addis Abeba, Ethiopia. pp. 126.

Edwards A.R. (1993). A non-colonizing aphid vector of potato virus diseases. Nature. London, 200:1233- 1234.

Evans L.T. (1997). Adapting and improving crops: the endless task. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 352:901-906.

FAOSTAT (2008). Potato world: Production and consumption. international year of the potato 2008 http://www.potato2008. Accessed date: 5-14-2012.

Hiskias Y., Lesemann D.E. and Vetten H.J. (1999). Occurrence, Distribution and Relative Importance of Viruses Infecting Hot Pepper and Tomato in the Major Growing Areas of Ethiopia

Hooker W. (1982). Virus diseases of potato. Technical Information Bulletin 19. International potato center, Peru. p20.

James W.C. (1974). Assessment of Plant Diseases & Losses. Annual Review of Phytopathology 12:27-48

Jones R.A.C., Charkowski A., Fribourg C.E., Stevenson R.W. and S.A. Slack S.A. (2009).

Potato Virus and Viruslike Diseases. In: Virus Diseases of Plants: Grape, Potato, and Wheat Image Collection and Teaching Resource CD-Rom. APS Press, St. Paul, MN 55121.

Kook-Hyung. (2001). Regulatory viral and cellular elements required for Potato Virus X replication. The Plant Pathology Journal 17:115-122.

Miha A.M., Rossel H.W. and Atiri G.I. (1993). Incidences and distributions of potato viruses in plateau state, Nigeria. African crop science journal. 2:131-138.

Muthomi J.W., Kinyungu T.N., Nderitu J.H. and Olubayo F.M. (2011). Incidence of Aphid transmitted viruses in farmer-produced seed potato tubers in Kenya. Afr. J. Hort. Sci 18-25.

Pankhurst R. (1964). Note on a history of Ethiopian Agriculture. Ethiopian observer 7:210-240.

Pourrahim R., Farzadfar, S., Golnaraghi, A.R. and Ahoonmanesh A. (2007). Incidence and Distribution of Important Viral Pathogens in Some Iranian Potato Fields. plant disease 91:609-615.

Salazar L.F. and Accatino P. (1990). The importance of potato virus diseases in developing countries. In: CIP.control of virus and virus like disease of potato and sweet potato Report of 3rd planning conference Lima, Peru. pp. 22-28.

Singh R. and Somerville T. (1992). Evaluation of the enzyme-amplified ELISA for the detection of potato viruses A, M, S, X, Y, and leafroll. American Journal of Potato Research 69:21-30.

Solomon-Blackburn R.M. and Barker H. (2001). Breeding virus resistant potatoes (Solanum tuberosum): a review of traditional and molecular approaches. Heredity 86:17-35.

Srinivasan R. and Alvarez J.M. (2007). Effect of Mixed Viral Infections (Potato Virus Y–Potato Leafroll Virus) on Biology and Preference of Vectors Myzus persicae and Macrosiphum euphorbiae (Hemiptera: Aphididae). Journal of Economic Entomology 100:646-655.

Ullman D.E., Cho J.J. and German T.L. (1991). Occurrence and distribution of cucurbit viruses in Hawaiian Island. Plant disease 75:367-375.

Valkonen (2007). Potato viruses: economical losses and biotechnological potential, Elsevier, Amsterdam, The Netherlands, 619-641. Accessed date 16-5-2012. http://www.scribd.com/doc/35336261/420/Viruses-Economical-Losses-and-Biotechnological-Potential.

Yusuf A.J., Sobh H. and Saad A. (2001). Incidence of potato virus diseases and their significance for a seed certification program in Lebanon. Phytopathol. Mediterr 40:113-118.