INCIDENCE OF POTATO VIRUSES AND BACTERIAL WILT DISEASE IN THE WEST AMHARA SUB-REGION OF ETHIOPIA

B. Bekele[1], E. Abate[2], A. Asefa[2] and M. Dickinson[3]

[1] *Ethiopian Institute of Agricultural Research, Plant Protection Research Centre, P.O. Box 37, Ambo, Ethiopia*
[2] *Amhara Region Agricultural Research Institute, P.O. Box 527, Bahar Dar, Ethiopia*
[3] *School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK*

SUMMARY

A survey of virus diseases and bacterial wilt was carried out in four major potato growing administrative zones in the west Amhara sub-region of Ethiopia in 2008. Leaf samples with symptoms suggestive of virus infection were collected from 38 randomly selected fields in 16 locations, whilst for bacterial wilt detection tuber and stem samples were collected from 23 and 12 fields in 15 and 12 locations, respectively. Disease incidences were visually assessed in the field and the identities of the pathogens were confirmed by laboratory testing using double antibody sandwich (DAS)-ELISA for viruses, and nitrocellulose membrane (NCM)-ELISA kits for *Ralstonia solanacearum*. In addition, an enrichment procedure was used to determine latent infection by *R. solanacearum*. Virus disease incidence varied from zero to 100% in different potato growing systems, whilst bacterial wilt incidence as high as 25% was recorded in farms in the west Gojam and north Gonder zones when assessed based on visual field symptoms. Results of laboratory testing for viruses confirmed the occurrence of at least five viruses, with *Potato virus S* (PVS) being the most widely distributed. Other viruses identified included *Potato virus X* (PVX), *Potato virus M* (PVM), *Potato leaf roll virus* (PLRV) and *Potato virus Y* (PVY), in order of importance. Mixed infections with two or more viruses were also detected. *Potato virus A* (PVA) was not detected in any of the samples tested. Latent infection by *R. solanacearum* was found in various potato fields, including experimental plots, farmers' seed potato production fields, suggesting the need to consider strict quarantine measure and restrict the free movement of seed tubers.

Key words: potato, Ethiopia, viruses, bacterial wilt, survey.

Corresponding author: B. Bekele
Fax: +251.11.2362325
E-mail: bekeleberhanu56@yahoo.com

INTRODUCTION

Ethiopia is among the top potato (*Solanum tuberosum L.*) producers in Africa, with 70% of its arable land in the high altitude areas above 1500 m being suitable for potato production (FAOSTAT, 2008). Currently, potato is produced mainly in the north western, central and eastern highlands of Ethiopia. The north western part of the country which mainly includes the highlands of the west Amhara sub-region (Fig. 1) is the major production area, and this region makes up over one third of the total area allotted to potato nationally. About 600,000 rural households are involved in potato production in the region, and according to CACC (2003) the area covered by potatoes in the region may be as high as 70,000 ha. The highlanders produce potato as a food security crop because of the limited crop choice that they have, whilst in the mid altitude areas potato is considered as an emergency crop as it is usually ready for consumption when the grain crops are not.

The national average productivity of potato in Ethiopia is 8 tons/ha, which is below the African continent average (10.8 tons/ha) (FAOSTAT, 2008). Diseases caused by viruses, bacteria and fungi are considered among the major biotic production constraints of potato. Symptoms suggestive of viral diseases are widely observed and distributed in the major production areas in the west Amhara sub-region. Evidence of the occurrence of potato viruses in Ethiopia was first reported in studies conducted in central, south and southeast Ethiopia during the 1984 and 1985 crop seasons (Agranovsky and Bedasso, 1985, 1986). The results of these consecutive studies indicated the presence of *Potato virus X* (PVX), *Potato virus S* (PVS), *Potato leafroll virus* (PLRV), *Potato virus Y* (PVY), *Potato virus A* (PVA) and *Potato virus M* (PVM). These studies, however, did not include the main production areas in the country such as the west Amhara sub-region, and the identity, distribution and status of viruses attacking potato have not been systematically studied using specific diagnostic methods. Studies made elsewhere indicate that yield losses as high as 90% can be incurred by viral diseases that can cause varietal degeneration (Cyperus and Bokx, 2005). In addition, symptoms of some potato viruses are often not apparent when in association with

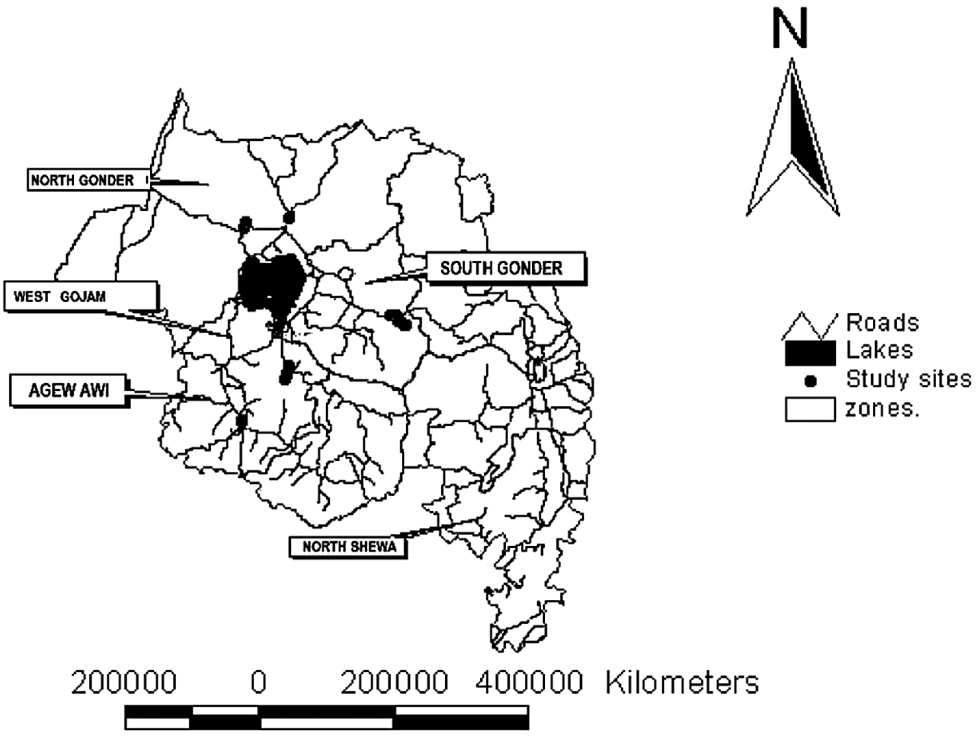

Fig. 1. Map of the west Amhara sub-region of Ethiopia from which samples were collected during the main season 2008. The four sampling zones of west Gojam, Agew Awi, north Gonder and south Gonder is shown, with the sampling sites marked.

the mosaics caused by PVX, PVY and PVS, so identification by visual observation of symptoms alone is not reliable (Fletcher *et al.*, 1996; Burrows and Zitter, 2005).

Bacterial wilt of potato (*Ralstonia solanacearum*) can also cause significant yield loss to potato (Ajanga, 1993). Because this pathogen stays in the soil for several years it prohibits subsequent production of potato in the same field. Moreover, this pathogen may stay latent without showing any symptoms in the field with the consequence of high impact on tuber yield in the upcoming season. Detection of latent infections by *R. solanacearum* requires sensitive diagnostic methods but, as yet, such methods have not been adopted in Ethiopia to inspect potato seed tubers and monitor the status of latent infection and its consequences. As the crop is vegetatively propagated, the diseases can easily be transmitted through tubers and cause very high economic losses across wide geographic areas.

This study was carried out to fill the current knowledge gap on identity, incidence and distribution of viral diseases and bacterial wilt of potato in the major production areas of the west Amhara sub-region with particular emphasis on emerging seed production schemes and germplasm evaluation work.

MATERIALS AND METHODS

Field visits and sample collection. An intensive survey and sample collection was conducted during the main rainy season of 2008 (from July 31–August 15, 2008) in selected representative major potato growing areas in four administrative zones of the west Amhara sub-region (Fig. 1). Samples for virus and bacterial wilt assay were collected from three systems: potatoes grown for seed, ware potato, and research plots in the west Gojam and north Gonder zones (Table 1). Sampling was done to meet three main objectives: typing of potato viruses occurring in the sub region, confirmatory test for bacterial wilt, and assessment of virus and bacterial wilt disease incidence and status.

Virus diseases. For virus typing, leaf samples with symptoms suggestive of virus infection were collected from about 1,460 plants in the surveyed areas. From each field, one to three composite samples were taken depending on the type and diversity of symptoms encountered, with each composite sample being a mixture of 10 individual plant samples. A total of 146 composite samples were collected from 38 fields in 16 locations, including experimental plots at the Adet Agricultural Research Centre (AARC) in west Gojam and the Gonder Agricultural Research Centre (GARC) in north Gonder. Leaflets were collected from the upper, middle and lower parts of at least 10 individual plants per field. Sampling was done at a constant interval depending on the distribution of crop in the respective administrative zones/locations surveyed following simple random sampling techniques and by moving diagonally across each

field. At the time of sampling crops were at the flowering-tuber setting stage. Disease incidence in the field was recorded visually as percent infection. Both symptomatic and non symptomatic samples were collected, but as the main aim of this survey was to type viruses occurring in the sub-region, emphasis was given to symptomatic plants, with plants showing different symptoms collected separately. Testing for six viruses was conducted in the tissue culture and serology laboratory of the Amhara Region Agricultural Research Institute (ARARI), Bahar Dar.

Bacterial wilt disease. Sampling for bacterial wilt in the field was performed following the simple random sampling strategy outlined for the viral diseases. Instead of leaves, in this case, tuber and stem samples were collected. In addition to random samples for testing latent infection, symptomatic plants with bacterial wilt-like symptoms were collected for confirmatory tests of *R. solanacearum* infection. Stem and tuber samples were mainly collected from released varieties in seed and ware potato producers' fields and in variety evaluation experimental plots at research stations. A total of 62 tuber and 29 stem samples were collected for laboratory testing in 23 and 12 fields from 15 and 12 locations, respectively. Emphasis was given to tuber sampling in all production systems to determine the level of infection, as tubers are the major source of inoculum as well as means by which the bacterium spreads from field to field and location to location.

Collected leaf, tuber and stem samples were labelled, put in plastic bags, taken to the laboratory and processed immediately or kept at 4-6°C in the refrigerator until processed for testing by DAS-ELISA and NCM-ELISA, respectively, for detection of viruses and bacterial wilt pathogens. During potato field inspections, data related to crop variables such as growth stage and variety, disease symptoms, disease incidences (%), purpose of production (ware, seed or research), altitude of each location and their corresponding geographical position using the geographical positioning system (GPS) were collected.

Laboratory tests. *Virus diseases*. All composite leaf samples were tested by DAS-ELISA (Clark and Adams,

Table 1. Virus disease incidence in potato as determined from symptomatic samples in growers fields and experimental plots during a survey in the west Amhara sub-region, Ethiopia, in 2008.

Zone	Farm type*	No. of Fields	No of Samples	[a]No. of fields / exp. Plots with incidences (%) of:							
				<5	5-15	16-25	26-35	36-45	46-55	56-65	>65
West Gojam	EP (VT-1)	1	18	4	7	0	0	0	5	0	2
	EP (VT-2)	1	8	0	0	1	4	0	1	0	2
	EP(VT-3)	1	11	2	3	1	1	0	0	0	4
	EP(VT-4)	1	14	1	5	4	3	1	0	0	0
	EP (VT-5)	1	20	15	2	2	1	0	0	0	0
	OSSI	1	15	4	3	6	1	0	0	0	1
	GH	1	5	1	0	0	0	0	0	0	0
	FF	3	3	0	1	2	0	0	0	0	0
	OFSI	6	6	3	0	1	2	0	0	0	0
	Sub total	16	100								
Agew Awi	OFSI	5	5	0	2	0	0	0	3	0	0
	Sub total	5	5								
North Gonder	EP (OFT)	1	3	0	3	0	0	0	0	0	0
	EP (VT-1)	1	10	4	3	1	1	0	0	0	1
	EP(VT-2)	1	14	5	4	4	0	0	0	0	1
	FF	4	4	1	3	0	0	0	0	0	0
	Sub total	7	31								
South Gonder	FF	8	8	5	3	0	0	0	0	0	0
	OFSI	2	2	1	1	0	0	0	0	0	0
	Sub total	10	10								
	Total	38	146								

*EP (VT 1-5) = Experimental plots sampled in different on-station variety trials at AARC and GARC; EP (OFT) = on-farm trial; OSSI = On-station seed increase; OFSI = Farmers potato seed production cooperative farm; FF = Farmers ware potato producers field; GH = Greenhouse samples.

[a]For experimental plots and OSSI, each sample represents a single plot of an experiment or seed increase plot and incidence was recorded per plot, whilst for other samples incidence was calculated per field.

1977) following standard protocols described in the International Potato Centre (CIP) DAS-ELISA kit instruction manual. DAS-ELISA kits were provided by the CIP Serology Laboratory at Lima (Peru). Each sample was tested for 6 viruses, namely *Potato leafroll viru*s (PLRV), *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato virus X* (PVX) and *Potato virus Y* (PVY).

Bacterial wilt. Stem and tuber samples were tested for *R. solanacearum* by NCM-ELISA. Sample preparation and serological tests were performed according to procedures outlined in the CIP NCM-ELISA kit instruction manual for detection of *R. solanacearum* in potato (Priou, 2001). Also this kit was supplied by the CIP Serology Laboratory. Tuber and stem samples with bacterial wilt symptoms were directly processed and tested by NCM-ELISA without enrichment. In addition, to increase the sensitivity of the test and detect latently infected plants, an enrichment procedure was carried out for non symptomatic plants in semi selective broth (modified SMSA) before performing NCM-ELISA. The enrichment was done by incubating the tuber and stem extracts prepared from non symptomatic samples in M-SMSA for 48 h at 30°C (Priou, 2001). In each of the tests, CIP positive (at concentrations of 10^6, 10^7, 10^8 bacteria/ml) and negative controls were used.

RESULTS

Viral and bacterial wilt diseases incidence in the field based on symptoms. The altitudes and geographical positions of surveyed areas were between 1,800 and 3,260 metres above sea level, and N10° 46.743-N12° 34.249 and E037° 027.690-E038° 30.823. The most commonly observed virus-like symptoms in potato were leaf curling, interveinal mosaic, mottling, reduced leaf size, deepening of leaf veins, narrow leaves and stunting. At the time of sampling, most plants were at the flowering-tuber setting stage, which is a good stage for symptom expression. Virus disease incidence assessed visually varied from zero to 100% in different farms and variety evaluation experimental plots (Table 1). The highest incidences for virus-like symptoms were commonly recorded in experimental plots. For instance, in variety trial-1 (VT-1) assessed at AARC, virus incidence as high as 100% was recorded on cv. Guassa planted as a standard check, whilst an incidence of 50% was recorded in two other varieties, Gudenie and Key Abeba (used as a local check). An incidence of 85% was also recorded in one of the test clones included in this variety trial. In variety verification trial (VT-2), disease incidence ranged from 25-70%, whereas in other variety development experiments (VT-3) evaluated, incidences of 100%, 80% and 80% were recorded, respectively, on three test clones. Low incidence (2.5%) was recorded on the standard check (cv. Jalene), while a test clone 'CIP 392640.539' was apparently free of any virus-like symptoms at the time of survey. When on-station seed increase (OSSI) plots of released potato varieties were visually evaluated, highest (>90%) incidence for virus-like symptoms was recorded in cv. Degemegn, and lowest incidence (2%) in cv. Tolcha. The other varieties had incidence of virus-like symptoms ranging from 15-30%.

On farmers' fields, disease incidence was less when compared to experimental plots. The highest incidence recorded was in the range of 50-60% in three out of five fields assessed around the Injibara area in the Tilili district of the Agew Awi zone on cv. Jalene. Farmers' fields inspected around Adet in the Yilmana Densa district (west Gojam) had highest virus disease incidences in the range of 20-30% (Table 1).

In north Gonder, disease incidence was between 10 and 15% on cv. Jalene planted at an on-farm fertilizer trial field at Chiliga. In the same location, 100% incidence was recorded on one of the clones included in the variety trial. Other test clones had incidences ranging between 2 and 30%. In another variety trial, the highest and lowest incidences were 75% and 5%, respectively. On the other hand, low disease incidences of 5-10% were recorded on farmers' fields planted with improved varieties in the north Gonder administrative zone. Low levels of virus-like symptoms were recorded in fields inspected in the south Gonder zone with common symptoms being deepening of veins and interveinal mosaic. The latter was particularly widely distributed in fields planted with local potato varieties.

With respect to bacterial wilt, plant wilting, browning of vascular tissue when cut and oozing of milky fluid from the vascular ring of cross-sectioned tubers were the most commonly encountered disease symptoms. Incidences as high as 25% were observed in localities around Adet Zuria in the Yilmana Densa district (west Gojam zone) and some farms in the Chiliga districts of the north Gonder administrative zone (Table 3).

Viral disease identity and incidence as determined by DAS-ELISA. DAS-ELISA testing of symptomatic plants indicated that out of the six viruses for which antibodies were provided, the occurrence of five viruses was confirmed in the west Amhara sub-region. PVA was not detected in any of the samples tested. Among the five viruses, PVS was the most widely distributed in the sub-region followed by PVX, PVM, PLRV and PVY (Table 2). In addition, the first two viruses (PVS and PVX) had high incidences compared to the other viruses identified. In most cases, simultaneous detection of two or more viruses was common, particularly in samples collected from experimental plots (Table 2). In addition, some uncommon symptoms such as narrow leaves were observed in some fields, which were assumed to be viral, but no virus could be detected by the antibodies used in this study.

Table 2. Detection of potato viruses by DAS-ELISA in samples collected from the west Amhara sub-region during the main rainy season, 2008.

Zone	District	Field types	No. of fields	No of samples	Viruses detected out of the samples collected and (percent)* PLRV	PVA	PVM	PVS	PVX	PVY
West Gojam	Adet	EP	5	71	43 (60.6)	0	38 (53.5)	60 (84.5)	40 (56.0)	20 (28.0)
		OSSI	1	15	2 (13.3)	0	5 (33.3)	6 (40.0)	2 (13.3)	2 (13.3)
		FF	3	3	1 (33.3)	0	0	2 (66.6)	0	0
		OFSI	6	6	2 (33.3)	0	1 (16.6)	4 (66.6)	4 (66.6)	1 (16.6)
	Bahir Dar	GH	1	5	0	0	3 (60.0)	4 (80.0)	1 (20.0)	1 (20.0)
Agew Awi	Tilili/Kosober	OFSI	5	5	0	0	0	5 (100)	2 (40.0)	0
North Gonder	Chilga	EP	3	27	12 (44.4)	0	16 (59.0)	16 (59.0)	6 (22.0)	0
		FF	4	4	0	0	1 (25.0)	4 (100)	0	0
South Gonder	Tach Gaint	OFSI	2	2	0	0	0	1 (50.0)	2 (100)	0
		FF	5	5	0	0	0	3 (60.0)	5 (100)	0
	Lai Gaint	FF	3	3	0	0	0	2 (66.6)	3 (100)	0
	Total		38	146	60 (41.0)	0	64 (43.8)	117 (80.1)	65 (44.5)	24 (16.4)

EP- Experimental plots; OFSI-farmers seed potato production field; OSSI- On station seed increase; FF- Farmers ware potato field planted with local cultivars, GH = green house samples.
*Figures in parenthesis indicate percent infection.

Table 3. Detection of bacterial wilt in symptomatic tuber and stem potato samples from the west Amhara sub-region in Ethiopia by direct NCM-ELISA without enrichment.

Symptomatic samples								
Zone	District/Locality	Altitude (m)	Variety	Field type sampled	Plant part sampled	Field incidence (%)	No of samples	NCM-ELISA Positive samples
West Gojam	Y/Densa-Goshiye	2626	Gera	OFSI	tuber	25	9	6 (66.7%)
	Y/Densa-Goshiye	2626	Gera	OFSI	Stem	25	15	13 (86.7%)
	Y/Densa -Adet	2205	Jalene	OFSI	Tuber	15	2	2 (100%)
	Y/Densa -Adet	2205	Local	FF	Tuber	5	1	0
North Gonder	Chilga	2254	Guassa	OFT	Stem	20	4	4 (100%)
	Sub total				Tuber		12	8 (66.7%)
					Stem		19	17 (89.5%)
	Total symptomatic samples						31	25 (80.7%)

OFSI -farmers' seed potato production field; FF- Farmers ware potato field planted with local cultivars; GH-Samples from green house potato; OFT – on-farm trial.

All the five viruses were detected in samples collected from experimental and on-station seed increase plots at AARC, and farmers' seed production fields in the vicinity of Adet in the west Gojam zone. The highest incidence was among samples collected from experimental plots (Table 2). In all the variety trials inspected at AARC, mixed infections of all the five viruses (PLRV, PVM, PVS, PVX and PVY) were recorded. In the north Gonder zone, four of the five viruses were recovered in samples collected from experimental plots at Chilga. In this area, PVS and PVM were equally identified in 59% of the samples. PLRV and PVX were identified from respectively, 48% and 22% of the samples collected in Chilga at on-farm experimental plots. Mixed infection of

one to four viruses were recorded in samples collected from experimental plots and farmers field in north Gonder. The most common virus combination was PLRV, PVM and PVS in four samples, while mixed infections of PVM, PVS and PVX; PLRV and PVS; PVM and PVS were each recorded in two samples. Combinations of PLRV, PVM, PVS and PVX; PLRV and PVM; PVS and PVX; PLRV and PVX were each detected in one sample. Two viruses (PVS and PVX) were detected in five samples collected from farmers' seed potato production cooperative fields at Enjibara and Tililli areas (Agew Awi zone). PVS was identified in all samples, while PVX was recovered in mixed infection with PVS from only 2 (40%) of the samples (Table 2).

In the south Gonder zone, leaf samples with symptoms suggestive of virus infection were collected from 10 fields (seven in Tach Gaint and three in Lai Gaint locations). The result showed that two (PVS and PVX) of the six viruses tested were detected, and all the tested samples were positive for PVX, with or without PVS. Of the total samples, 6 of 10 were PVS-infected. Mixed infections of PVS and PVX were detected in six out of 10 samples. Most of the samples tested from south Gonder were collected from farmers' fields planted with local varieties indicating wider distribution of PVS and PVX in local potato production systems as well (Table 2).

Bacterial wilt incidence and distribution as determined by NCM-ELISA. A total of 31 symptomatic plant samples (19 stem and 12 tuber samples) were collected for confirmatory testing of *R. solanacearum* infection (Table 3). All tuber samples collected from farmers' seed potato production cooperative farms planted with cvs Jalene at Adet gave positive reactions to the pathogen. Similarly, at the same location, 86.7% of the stem samples and 66.7% of the tuber samples collected from farmers' seed increase plots planted with cv. Gera tested positive. All the stem samples collected from symptomatic plants at the Chilga on-farm experimental plots were found positive when tested by NCM-ELISA.

In addition to symptomatic samples, a post enrichment NCM-ELISA test was carried out for non-symptomatic (apparently healthy looking) potato plants to detect latent infection by *R. solanacearum* (Table 4). Of the total of 50 tuber and 10 stem samples tested, *R. solanacearum* was detected in 11 (18.3%) out of 60 samples. As shown in Table 4, of the five administrative zones sampled, the bacterium was detected in all except south Gonder. Latent infection by *R. solanacearum* was recovered from both the local and improved varieties that were tested.

Table 4. Detection of bacterial wilt in random asymptomatic tuber and stem potato samples from the west Amhara sub-region in Ethiopia by enrichment NCM-ELISA.

Random samples							
Zone	District/Locality	Altitude (m)	Variety	Field type sampled	Plant Part Sampled	No. of samples	Positive samples
West Gojam	Adet	2400	Siquare(Local)	FF	tuber	2	0
		2626	Jalene	OFSI	tuber	2	0
		2400	Sisay (Local)	FF	tuber	1	0
		2205	Zengena	OSSI	tuber	1	0
		2626	Gera	OFSI	stem	5	2
		2626	Gera	OFSI	tuber	30	6
	Bahir Dar-ARARI	1800	Zengena	GH	tuber	1	0
		1800	Guassa	GH	tuber	1	0
Agew Awi	Enjibara	2503	Jalene	OFSI	tuber	2	0
		2503	Deme (Local)	FF	tuber	1	0
		2503	Samuni (Local)	FF	tuber	1	1
North Gonder	Chilga	2254	Chilga local	FF	tuber	1	0
		2254	Guassa	EF	stem	2	1
		2254	Guassa	EF	tuber	2	1
South Gonder	Tach Gaint	2892	Kara (Local)	FF	tuber	1	0
		3260	Jalene	OFSI	tuber	3	0
		3260	Jalene	OFSI	stem	3	0
	Lai Gaint- Gob gob	3054	Local	FF	tuber	1	0
	Sub total				Tuber	50	8 (16%)
					Stem	10	3 (30%)
	Total random samples					60	11 (18.3%)

OFSI -farmers' seed potato production field; OSSI- On station seed increase; FF- Farmers ware potato field planted with local cultivars; GH-Samples from green house potato.

DISCUSSION

From this survey in the west Amhara sub-region of Ethiopia, PVS was the most frequently identified and distributed among six potato viruses tested in respective zones and across zones studied, followed by PVX, PVM, PLRV and PVY. Mixed infections with two or more viruses were also commonly detected, among which PVS and PVX combination was recorded in 12 samples. This finding correspond with the survey results conducted in central, south and southeast Ethiopia during the 1984/85 seasons (Agranovsky and Bedasso, 1985, 1986) that reported PVS and PVX as the most common viruses identified with PLRV, PVY, PVM and PVA being less widely distributed in the regions surveyed. However, PVA was not identified in any of our samples collected in the west Amhara sub-region. Causing a mild mosaic, PVS is the most frequently found virus in potato worldwide and is very contagious (Cyperus and Bokx, 2005). Infection rates of 100% have been reported from many countries (Cyperus and Bokx, 2005), which agrees with the present results. It is also known that infection by PVS may result in yield losses of up to 20%, but higher losses can be incurred if infection is combined with PVX. Being the second and third most widely distributed viruses next to PVS, PVX and PVM cause mild symptoms and bring about low yield loss, however, they are reported to cause significant impact on potato yield when in combination with PVS and other viruses. PLRV was the fourth most widely distributed virus, and Cyperus and Bokx (2005) indicated that yields of plants with secondary infection of PLRV are often reduced by more than half; while in highly sensitive varieties yield loss can be as much as 90%. As mixed infections of two or more of these viruses were recorded in many of the locations surveyed, one can assume that potato farmers are facing heavy yield losses every year, and evidence for this comes from the high disease severity and incidence observed in some farmers' fields and experimental plots at research stations.

Some solanaceous plant hosts, weeds (*Chenopodium amaranticolor, Datura metel,* and *Datura stramonium)* and plants belonging to other families such as *Nicotina* spp. and *Phaseolus vulgaris* are commonly grown in the study area, and are alternate hosts for some of the viruses detected (Kook-Hyung, 2001), contributing to the widespread occurrence of these viruses along with the presence of aphid and biting insect vectors. Another possible factors to account for the widespread and high virus disease incidences may be the overlapping potato growing seasons and lack of a seed potato health testing program.

The presence of different viruses at a higher incidence rate on variety trials under research managed experimental plots than farmer's fields and on-farm seed increase plots seems paradoxical as far as the high level of management in research stations is concerned vis-a-vis farmers' poor management. However, this result seems to have been associated with the presence of susceptible clones in the test materials and/or because of high inoculum build up in the experimental stations over years without break. Rarely encountered symptoms in some potato experimental fields sampled such as narrow leaves, which were suspected to be caused by viral infection, did not give positive reaction when tested against antibodies for the six viruses. This may indicate the presence of other viruses or virus-like organisms that could not be detected by the reagents used in these tests, which calls for further investigation.

One of the objectives of using NCM-ELISA was a confirmatory test whether symptomatic potato plants are caused by bacterial wilt or not. In the direct NCM-ELISA test (Table 3), of the total of 12 tuber and 19 stem composite samples collected from symptomatic plants, *R. solanacearum* was recovered from 8 (66.7%) tuber and 17 (89.5%) stem samples, respectively. Overall, *R. solanacearum* was detected in 25 (80.7%) of the 31 tuber and stem symptomatic samples. From this result it is evident that there is high chance that most of the potato plants with wilting symptoms are infected by *R. solanacearum*. Only six (19.4%) out of the 31 samples tested were negative. Priou (2001) reported that post enrichment NCM-ELISA can detect as few as 10 bacteria per ml of extract instead of 10^6-10^7 bacteria/ml without enrichment. In this study, the negative reaction of some symptomatic samples when tested by NCM-ELISA without the enrichment procedure may be attributed to low bacterium concentrations in the extracts, or due to infection of plants by other soil-borne pathogens and/or insects that could cause wilting symptoms similar to *R. solanacearum,* and this probably resulted in false negatives. However, had it not been for shortage of reagents during this study, it would have been imperative re-testing and confirming all symptomatic samples that were negative by using post enrichment ELISA. This therefore suggests the need for paying particular attention while collecting and rating bacterial wilt disease incidence in the field and emphasizes the importance of using efficient detection methods such as post enrichment NCM-ELISA.

When 60 random samples (50 tuber and 10 stem samples) collected from apparently healthy looking plants were tested by post enrichment NCM-ELISA for latent infection, *R. solanacearum* was recovered from 11 (18.3%) of the samples, both from improved and local varieties in different farms as well as in tuber and stem samples at higher altitudes over 2,500 m above sea level. This result supports findings by Ciampi *et al.* (1980) and Hayward (1991) who have confirmed latent infection of *R. solanacearum* in tropical cool conditions at altitudes above 2,500 m, and that of Janse (1996) who has indicated that bacterial wilt has become a serious threat

to potato seed production in cool, temperate countries of northern Europe. This level of latent infection is high, particularly considering that bacterial wilt is a quarantine disease of zero tolerance level in seed tuber production (Priou *et al*., 1999b).

Interestingly, assuming that bacterial wilt is a quarantine disease of zero tolerance level in seed tuber production, higher levels of latent infection (20%) were recorded in samples collected from potato seed tuber production fields at altitudes of 2,626 m above sea level (Table 3). These potato seed tubers were meant to be distributed to growers for use as a planting material in the subsequent growing season, indicating the potential danger of using potato seed tubers from such infected fields as planting materials in the upcoming season. As suggested by Nortje (1997) and Kakuhenze *et al*. (2000), this is a consequence of a lack of rigorous seed health testing and a certification programme, which is also one of the major drawbacks within the potato seed production system in the area. This drawback may in part be associated with the lack of technical capacity, facilities and availability of affordable and efficient detection methods. The classical detection method of tuber infection is time-consuming (requiring tuber incubation for three to four weeks at 30°C) and space-consuming and may not reveal low infection rates, whereas post enrichment NCM-ELISA can have the combined advantages of low-cost (about $0.30/sample for supplies), ease, and speed (6 h after enrichment of the extracts), and does not require extensive laboratory equipment (Priou *et al*., 1999a). Therefore, the use of NCM-ELISA with the post enrichment procedure can be recommended as a powerful tool for efficient and economical detection of latent infection by *R. solanacearum* for routine use in quarantine procedures, seed certification and quality testing, as well as in assessing susceptibility of breeding lines to bacterial wilt in experimental stations in the Amhara region and other potato growing regions in the country.

Results of this study have shown comparably high levels of latent infection of *R. solanacearum* in both stem and tuber samples tested, indicating the potential for using stem sampling as an alternative to tubers, since tuber sampling has economic implications. Mwangi *et al*. (2008) observed similar results and found a positive correlation between stem and tuber testing. We recommend use of stems as an alternative sampling to tubers in seed health testing programmes.

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