Enzyme linked immune-sorbent assay based survey on bacterial wilt and viral diseases of potato in western Amhara Region

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Abstract

In the Amhara region, spread of improved potato varieties is challenged by incidence of wilt and viral diseases. Nonetheless, no attempt has, so far, been made to establish their identity through laboratory procedures. In order to bridge this gap, a survey was conducted on potato viral and bacterial wilt diseases from July 31 to August 15, 2008 in selected potato growing areas of Western Amhara region. A total of 146 composite leaf samples were collected and tested for six viruses: PLRV, PVA, PVM, PVS, PVX and PVY using DAS-ELISA. Similarly, a total of 62 tuber and 30 stem samples were collected mainly from released potato varieties in farmers' fields and tested for bacterial wilt pathogen, *Ralstonia solanacearum* (Rs) by NCM-ELISA. Results showed that all the viruses but PVA showed positive reaction. PVS was the most prevalent viral disease followed by PVX, PVM, PLRV and PVY in that order. Likewise, most of the wilted plants checked for bacterial wilt were found positive indicating that the pathogen is a likely cause of wilting. In addition, healthy samples tested by the enrichment procedure appeared positive for some of the localities. Generally, the existence of these diseases means that they can incur very high yield loss on potato. Especially, control of bacterial wilt is very difficult and the yield loss is unbearable. The pathogen can stay in soil for several years and can be transmitted by infected seed, irrigation water and farm implements. It can also restrict production of several other crops related to potato. Hence, all possible measures have to be taken in order to limit expansion of these diseases.

Key words: Bacterial wilt, DAS-ELISA, NCM-ELISA, potato, viral disease.

Introduction

Potato (*Solanum tuberosum* L.) is one of the widely produced and consumed horticultural crops in the western Amhara Region. Potato production in the region reaches as high as 70, 0000 ha of which 95% comes in the western part of the region (CSA, 2003). Amhara Region contributes over one third of the total area allotted to potato production in Ethiopia. About 600,000 rural households are engaged in potato

production in the region. Its production is mainly restricted to the highlands where there is limited crop choice, and is one of the major food security crops. Nevertheless, the average productivity of potato in the region is much lower than the national (7.2 t ha^{-1}) and African (10.8 t ha^{-1}) average (FAO, 2008). However, research results in the region indicated that productivity of 30 t $ha^{-1}$is achievable on research plots using quality seeds of improved varieties and improved production packages.

The existing potato seed system is characterized by traditional informal multiplication and distribution of seed tubers by small scale subsistent farmers. Consequently, poor yielding local potato cultivars that are highly susceptible to diseases dominate the system. Efforts are, however, underway to multiply and distribute improved potato varieties. Nonetheless, these attempts are increasingly challenged by incidence of wilt and viral diseases. Indeed, potato diseases and lack of quality seed tubers are two of the major potato production constraints in the area. However, there has not been any systematic scientific study made to establish the identity, incidence and distribution of potato diseases. Knowledge of identity, incidence and distribution of diseases could help to design timely and appropriate management strategies. This is particularly important in potato where because of its vegetative mode of propagation diseases can easily be transmitted through tubers and cause very high economic loss across wider area. In potato viral disease symptoms are often masked by the simultaneous occurrence of mosaics caused by potato virus X (PVX), potato virus Y (PVY) and potato virus S (PVS). This makes identification of viral diseases based only on symptom difficult (Fletcher *et al*., 1996; Burrows and Zitter, 2005). On the other hand, bacterial wilt (brown rot) caused by *Ralstonia solanacearum* can stay latent without showing any symptom. This study was, therefore, undertaken to establish the identity, level of incidence and distribution of viral and bacterial wilt diseases of potato in the major production areas of western Amhara Region.

Materials and methods

Field visit and sample collection

An extensive potato viral and bacterial wilt diseases survey and sample collection was conducted from July 31 to August 15, 2008 in selected potato growing areas of four administrative zones of west Amhara Region (Figure 1; Table 1).

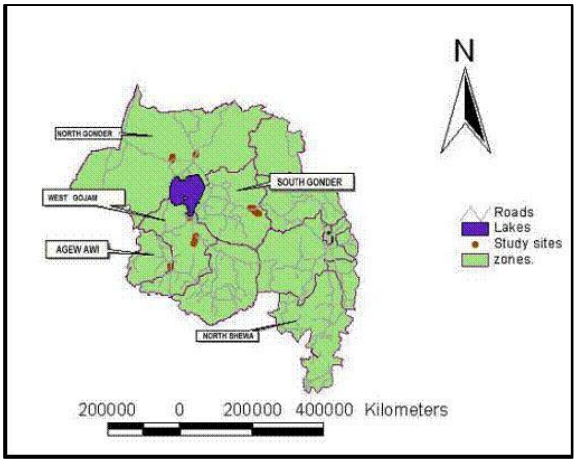

Figure 1. Map of Amhara Region with the four sampling zones. Sampling sites are marked with red dots.

Virus diseases: For typing viruses infecting potato, leaf samples with symptoms suggestive of virus infection were collected in the surveyed areas. From each field, one or more composite samples were collected depending on the type and diversity of symptoms encountered, with each composite sample tested being a mixture of 100 individual plant samples. A total of 146 composite samples were collected from 37 fields located at 16 sites, including experimental plots at Adet and Gonder Agricultural Research Centres (Table 1). Leaflets were collected from upper, middle and lower parts of the individual plants sampled. Sampling was done at a constant interval depending on the distribution of the crop in respective zones/locations surveyed. Simple random sampling of symptomatic plants was made by making a transverse walk across the field. Disease incidence was recorded visually as percent infection.

Bacterial wilt: Bacterial wilt field sampling was done following simple random sampling as outlined for the viral diseases. However, in this case, tuber and stem samples were collected.

Table 1. Potato samples collected and tested for potato viruses and bacterial wilt diseases from four zones of western Amhara Region.

Administrative Zones	Leaf samples			Tuber samples			Stem samples		
	Number of samples	Number of fields surveyed	Number of locations	Number of samples	Number of fields surveyed	Number of locations	Number of samples	Number of fields surveyed	Number of locations
West Gojjam	100	16	4	51	13	8	11	1	1
North Gonder	31	6	4	3	2	2	6	3	3
Awi	5	5	2	4	4	2	10	5	5
South Gonder	10	10	6	4	4	3	3	3	3
Total	146	37	16	62	23	15	30	12	12

Sampling was done mainly from released potato varieties in farmers' fields to determine the status of disease incidence and distribution. Accordingly, a total of 62 tuber and 30 stem samples were collected and tested from 15 and 12 locations in 23 and 12 fields, respectively (Table 1).

In addition, data on crop growth stage and variety, disease symptoms, diseases incidences (%), purpose of production (ware or seed), and altitude of each location and its corresponding geographical position (using geographical positioning system) were collected during potato field inspection. The collected leaf, tuber and stem samples were labelled, put in plastic bags, brought to laboratory and kept at 4-6 ^{O}C in refrigerator until processed for detection test of viruses and bacterial wilt pathogens.

Laboratory test

Virus diseases: Leaf samples were tested for six viruses, namely potato leaf roll virus (PLRV), potato virus A (PVA), potato virus M (PVM), potato virus S (PVS), potato virus X (PVX) and potato virus Y (PVY) using Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) following the International Potato Centre (CIP) DAS-ELISA kit standard protocol described in CIP instruction manual (CIP, 2001). Batteries of six polyclonal antibodies developed against six viruses were used.

Bacterial wilt disease: Stem and tuber samples were tested for bacterial wilt pathogen, *Ralstonia solanacearum (Rs),* by Nitrocellulose Membrane-Enzyme Linked Immunosorbent Assay (NCM-ELISA). In addition to random samples t□ t d

Results and discussion

Viral and bacterial wilt diseases incidence in the field

The most commonly observed virus-like symptoms in potato were leaf curling, interveinal mosaic, mottling, reduced leaf size, leaf vein deepening and stunting. Disease incidence varied from none to 100% infection in different farms and variety evaluation plots. For instance, in a national variety trial (NVT) at Adet Agricultural Research Center (AARC), 100% incidence was recorded on variety *Guassa* used as standard check (Figure 2) and more than 50% incidence on *Gudenie* variety and the local check. Among test clones included in NVT, the highest incidence (85%) was recorded on CIP 395112.36. In a variety verification trial (VVT), disease incidence ranged from 25-70%. In a regional variety trial (RVT)-III, 100% infection was recorded on a clone "CIP 392640.516" and 80% infection on other two clones, CIP 391588.563 and CIP 392640.504. Some clones in the variety evaluation plots looked apparently free of any symptom. When seed increase plots of released potato varieties were visually evaluated, highest (> 90%) incidence was recorded on variety *Degemegn*.

In north Gonder zone, disease incidence of 10-15% was recorded on *Jalene* variety used for on-farm fertilizer trial by the Gonder Agricultural Research Center (GARC) at Chiliga. In the same location, 100% incidence was recorded on one of the clones included in RVT-III while lower incidences ranging between 2% and 30% were recorded on the rest of test clones. In RVT-I, highest and lowest incidences were 75% and 5%, respectively. Again, low disease incidence of between 5% and 10% was recorded on farmers' fields planted with improved varieties. Most plants were at flowering stage at the time of sampling which is an appropriate time for symptoms expression. In South Gonder zone, the most common symptoms were deepening of veins and interveinal mosaic. The latter was particularly more prevalent in farmers' fields planted with local potatoes.

Figure 2. Viral disease incidence on *Guassa* potato variety in germplasm evaluation plots.

With regard to bacterial wilt, most common symptoms were: wilting (Figure 3), browning of vascular tissue upon cutting and oozing milky fluid from the vascular ring of cross-sectional cut tubers. Bacterial wilt incidence of as high as 20-25% was recorded in localities surrounding Adet in Yilmana Densa district of West Gojjam Zone and some farms in Chiliga districts of North Gonder Zone.

Figure 3. Wilted symptomatic plants tested almost 100% positive for bacterial wilt of potato (*Ralstonias solanacearum*).

Viral diseases identity and incidence as determined by DAS ELISA

DAS-ELISA testing of symptomatic plants indicated that five out of the six viruses tested gave positive reaction (Table 2). Potato virus A was not detected in any of the samples tested. Potato virus YS (PVYS) was the most widely distributed viral disease followed by PVX, PVM and PLRV and PVY in that order.

Table 2. DAS-ELISA results of samples collected from the surveyed areas.

Zone	District	Sample Field	No of Samples	Virus Detected as % of the samples collected					
				PLRV	PVA	PVM	PVS	PVX	PVY
W. Gojjam	Adet	EF	71	60	0	53	84	56	28
		OSSE	10	20	0	10	40	20	20
		FF	5	20	0	0	80	0	0
		OFSE	9	33	0	11.1	55.5	22.2	11.1
	Bahir Dar	ARARI	5	0	0	0	20	0	20
Awi	Tilili/Kosober	OFSE	5	0	0	0	100	40	0
N. Gonder	Chilga	EF	27	48	0	59	59	22	0
		OFSE	4	0	0	25	100	0	0
S.Gonder	Tach Gaint	OFSE	2	0	0	0	50	100	0
		FF	5	0	0	0	60	100	0
	Lai Gaint	FF	3	0	0	0	66.6	66.6	0

EF = Experimental fields, OSSE = On farm seed increase, OSSE = On station seed increase, FF = Farmers ware potato field planted with local cultivars.

Potato virus S and PVX had also high incidence percentage as compared to the rest of the diseases. In most of the cases, simultaneous detection of two or more viruses was common particularly in experimental plots. Disease incidence appeared very high at Adet experimental station where 100% incidence was recorded for some of the varieties in variety evaluation fields. Similarly, all viruses but PVA were detected in on-station and on-farm seed increase fields at and in the vicinity of Adet.

In North Gonder Zone, all the five viruses that were tested positive at Adet experimental plots were detected from experimental plot samples at Chilga with 59% for PVS and PVM, 48% for PLRV and 22% for PVX. However, from the on-farm seed increase plots only PVS and PVM were detected with incidence levels of 100% and 25%, respectively. Out of five samples collected from on-farm seed increase plots at Kosober and Tilili areas, only PVS and PVX were detected. Potato virus S was identified from 100% of the samples and PVX was from 40% of the samples. In South Gonder, two (PVS and PVX) of the six viruses were detected on symptomatic leaf samples collected from 10 locations (7 in Tach Gaint and 3 in Lai Gaint), and all the tested samples were positive to either one or two of these viruses. Of the total samples, 6 (60%) were positive for PVS, while PVX was detected in all of the samples collected from 10 locations. Mixed infections of PVS and PVY were detected in 6 (60%) out of 10 samples. Most of the samples collected from farmers' fields cropped with local varieties in South Gonder indicated a wider distribution of PVS and PVX in the local potato production system. Simultaneous detection of viruses in the samples tested suggests that there is high yield loss or at least risk of high yield loss due to interaction of the different viral pathogens detected in the samples. Such phenomenon is substantiated by several workers. Cyperus and Bokx (2005) reported that concurrent occurrence of PVS and PVX causes much higher yield loss than their independent occurrence. Similarly, Burrows and Zitter (2005) reported heavier yield loss on co-occurrence of PVY and PVX or PVA. Simultaneous infection of potato plants by different viruses also hastens the rate of varietal degeneration manifested by a decrease in vigor, productivity and resistance to diseases of potato (Sangar *et al*., 1988). According to Cyperus and Bokx (2005), simultaneous occurrence of PLRV and PVY are the most important cause of 'potato degeneration'. Besides, the study showed that some uncommon

seemingly viral infection symptoms (Figure 4) did not appear to have positive reaction which might indicate presence of other viral or viral like organisms that were not included in the testing kit.

Figure 4. Virus-like symptoms.

Generally, the study showed that viral incidence is higher on experimental plots as compared to farmers' fields (Table 2). This can be explained by the presence of susceptible clones in the test materials and/or because of high inoculums build up in the experimental stations or their facilities over time.

Bacterial wilt incidence and distribution as determined by NCM ELISA

Thirty one symptomatic samples (19 stem and 12 tubers) that showed wilting symptom were taken from on-farm seed increase plots planted to variety *Jalene* at Adet and tested for *Ralstonia solanacearum* infection by NCM-ELISA technique (Table 3). The result indicated that all the tuber samples and 86.6% of the stem samples tested positive to the pathogen. Similarly, all the stem samples collected from experimental plots at Chilga tested positive.

On the other hand, samples taken from tubers of wilted local cultivar at Adet tested negative for the pathogen suggesting that the causative agent might be something else. The finding suggested that *Ralstonia solanacearum* is the likely causative agent for potato plant wilting in the surveyed areas. Unfortunately, most of the sampled fields that tested positive were planted to improved varieties for seed production. These fields are managed by those who have no awareness about the disease and neither were they supervised by those who

have the expertise. This scenario signifies a very high risk that could be induced by such schemes. In addition, 50 tuber and 10 stem samples were randomly collected from non-symptomatic plants from fields planted to improved varieties for seed and local cultivars for ware purpose.

Table 3. Confirmatory test of symptomatic /wilted/ plants without enrichment procedure of NCM-ELISA.

Zone	District/Locality	Variety	Plant Part Sampled	No of samples	NCM-ELISA Positive (%)
W.Gojjam	Y/Densa - Goshiye	Gera	Tuber	9	66.6
	Y/Densa - Goshiye	Gera	Stem	15	86.6
	Y/Densa -Adet	Jalene	Tuber	2	100.0
	Y/Densa -Adet	Local	Tuber	1	0.0
N.Gonder	Chilga	Guassa	Stem	4	100.0

The NCM-ELISA test was run through the enrichment procedure (Figure 5) so that latent infection of small pathogen load can easily be detected. Positive (10^6, 10^7, 10^8) and negative controls from CIP were used for comparison. The result confirmed the existence of *Ralstonia solanacearum* in latently infected and healthy looking field sampled plants from Adet, Chilga and Injibara (Table 4). Also a sample collected from local potato cultivar field tested positive. The danger of latent infection is that the pathogen can be multiplied unnoticed and disseminated to wider area risking potato and other related species production. On the other hand, none of the samples collected from South Gonder tested positive for *Rs*.

Figure 5. NCM ELISA (enrichment procedure) test result of samples collected from improved potato varieties grown for seed production.

Table 4. Test results of potato plants through the enrichment procedure of NCM-ELISA.

Zone	District/Locality	Variety	Type of field sampled	Plant part sampled	No of samples	NCM-ELISA positive (%)
W.Gojjam	Adet	Local Siquare	FF	tuber	2	0
		Jalene	OFSE	tuber	2	0
		Local Sisay	FF	tuber	1	0
		Zengena	OSSE	tuber	1	0
		Gera	OFSE	stem	5	40
		Gera	OFSE	tuber	30	20
	Bahir Dar-ARARI	Zengena	OSSE	tuber	1	0
		Guassa	OSSE	tuber	1	0
Awi	Enjibara	Jalene	OFSE	tuber	2	0
		Local (Deme)	FF	tuber	1	0
		Local (Samuni)	FF	tuber	1	100
N.Gonder	Chilga	Chilga local	FF	tuber	1	0
		Guassa	EF	stem	2	50
		Guassa	EF	tuber	2	50
S.Gonder	Tach Gaint	Local (Kara)	FF	tuber	1	0
		Jalene	OFSE	tuber	3	0
		Jalene	OFSE	stem	3	0
	Lai Gaint-Gob gob	Local	FF	tuber	1	0

EF = Experimental fields, OSSE = On farm seed increase, OSSE = On station seed increase, FF = Farmers ware potato field planted with local cultivars.

Conclusion and Recommendations

The study confirmed the existence of at least five viruses attacking potato in Western Amhara Region. Potato virus S appeared the most commonly detected and widely distributed followed by PVM, PLRV, PVX and PVY in that order. Potato virus A was not detected in any of the samples tested. In most of the cases, 2 or more viruses were simultaneously detected indicating their high potential to cause substantial yield loss. The use of DAS- and NCM-ELISA proved very powerful diagnostic tools to inspect potato planting materials prior to distribution to growers to ensure production of clean planting

materials. The level of viral disease incidence was higher on visual observation. However, this has not been reproduced in laboratory tests. This might indicate the presence of other pathogens with closer symptom to the identified ones which could not be confirmed in this study because of lack of kits. Alternatively, it can be a manifestation of some form of nutrient deficiency. The improved informal seed system judges the health of seed tubers based on physical symptomatic expression. This procedure is not valid for latent viral and bacterial diseases. Therefore, whenever there are doubts on the health standard of the plants testing incidence of important diseases should be carried out following appropriate pathological procedures.

Similarly, bacterial wilt was detected in tuber and stem samples collected from symptomatic (wilting) and apparently healthy looking plants, indicating the powerfulness of NCM-ELISA in detecting latent infection in healthy looking plants. Despite the relatively small number of samples analyzed, incidence of bacterial wilt is confirmed on substantial proportion of the samples collected across the surveyed areas. As there is no remedial for this disease, the high incidence of latent infection indicates the potential danger that potato production could face in the region unless appropriate precautionary measures are taken. Should bacterial wilt disease is effectively prevented strict adherence to preventive measures is essential. As the disease is mainly transmitted through seed, farm implements and irrigation water, awareness has to be created among various stakeholders in major potato growing areas of the region. Hence, imposing quarantine law, and potato seed movement restriction from location to location should be strictly implemented to minimize wide spread of the disease. Moreover, integrating the use of healthy planting materials with appropriate cultural practices such as crop rotation with non solanaceous crops should be sought and taught to farmers as economical, cost effective and safe control measures. Transport, marketing and utilization of seed tubers for table potato production should be supported by certificate. In addition, the regional plant health clinic laboratories have to frequently monitor incidence and distribution of selected diseases and provide information. In addition, monitoring populations of important potato disease vectors has to be made on areas specialized for seed potato production.

The regional research system has been and is striving to generate and supply improved potato technologies to farmers. Nonetheless, such efforts did not have the required support from laboratories in terms of pathogen testing and availing healthy starter planting material. Hence, the high disease incidence recorded in the germplasm evaluation plots should not be surprising. Since the research centres are the only source of planting material for improved varieties, high disease incidence in experimental plots implies the likelihood of disease incidence on farmers' plots is high. The high disease pressure in research plots can also hamper genetic expression of the genotypes and thereby lowers the chance of developing improved varieties by the research system. Therefore, it is imperative that the tissue culture laboratory of the Amhara Region Agricultural Research Institute be part and parcel of the research endeavor so as to furnish healthy starter planting material both for germplasm evaluation as well as seed production.

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References

Burrows, M.A. and Zitter, T.A. 2005. Virus problems of Potatoes; Vegetable MD Online. USDA-ARS and Department of Plant Pathology, Cornell University, Ithaca. Accessed on 8/10/2008.

CSA (Central Statistics Authority) (2003). Agriculture sample survey 2002/2003: Report on area and production for major crops. Statistical bulletin 200, CSA, Addis Abeba, Ethiopia.

Cyperus, C. and Bokx, J.A. 2005. Virus Diseases. In: Mulder and Turkensteen, L.J. (eds) Potato Diseases. Aardappelwereld B.V. and NIVAP, Den Haag The Netherlands.

FAO. 2008. Potato world: Production and consumption. International year of the potato 2008.

Fletcher, J.D., Lewthwaite, S.L., Boddington, H.J., Nott, H.M. and Wood, R.J. 1996. Virus diseases survey of ware potato crops. *New Zealand Journal of Crop and Horticultural Science* 24: 7-12.

CIP (International Potato Center). 2001. CIP DAS-ELISA kit for potato viruses detection: Instruction manual. Serology Laboratory, International Potato Center. Lima, Peru.

Priou, S. 2001. CIP NCM-ELISA kit for the detection of *Ralstonia solanacearum* in potato: Instruction for use. International Potato Center (CIP), Lima, Peru.

Sangar, R.B.S., Agrawal, H.O. and Nagaich, B.B. 1988. Studies on the translocation of potato viruses X and Y in potatoes. *Indian Phytopathology* 41: 327-331.