World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:12, No:12, 2018

Survey of Potato Viral Infection Using Das-Elisa Method in Georgia

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Abstract**—**Plant viruses can cause loss of yield and quality in a lot of important crops. Symptoms of pathogens are variable depending on the cultivars and virus strain. Selection of resistant potato varieties would reduce the risk of virus transmission and significant economic impact. Other way to avoid reduced harvest yields is regular potato seed production sampling and testing for viral infection. The aim of this study was to determine the occurrence and distribution of viral diseases according potato cultivars for further selection of virus-free material in Georgia. During the summer 2015-2016, 5 potato cultivars (Sante, Laura, Jelly, Red Sonia, Anushka) at 5 different farms located in Akhalkalaki were tested for 6 different potato viruses: Potato virus A (PVA), Potato virus M (PVM), Potato virus S (PVS), Potato virus X (PVX), Potato virus Y (PVY) and potato leaf roll virus (PLRV). A serological method, Double Antibody Sandwich-Enzyme linked Immunosorbent Assay (DAS-ELISA) was used at the laboratory to analyze the results. The result showed that PVY (21.4%) and PLRV (19.7%) virus presence in collected samples was relatively high compared to others. Researched potato cultivars except Jelly and Laura were infected by PVY with different concentrations. PLRV was found only in three potato cultivars (Sante, Jelly, Red Sonia) and PVM virus (3.12%) was characterized with low prevalence. PVX, PVA and PVS virus infection was not reported. It would be noted that 7.9% of samples were containing PVY/PLRV mix infection. Based on the results it can be concluded that PVY and PLRV infections are dominant in all research cultivars. Therefore significant yield losses are expected. Systematic, long-term control of potato viral infection, especially seed-potatoes, must be regarded as the most important factor to increase seed productivity.

Keywords**—**Diseases, infection, potato, virus.

I. Introduction

POTATO is one of the demanded cultivars in Georgia. The mountain regions (Akhalkalaki and Tsalka) of Georgia have the best climatic conditions for potato seed production [1]. High quality seed is directly associated with virus free potato materials. Viral contamination is considered as the main problem in seed potato production system [2]. Potato viruses: PVA, PVM, PVS, PVX, PVY and PLRV are widely spread and can cause significant economic losses (50-80%) [3]. Plant virus transmission can be mechanical through wounds, by a biological intermediary or both depending on the variety [4] Potato virus are transmitted most quickly from mixed-strain infection sources [5].

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PVY is the most severe infection compared to others [6]. PVY can infected family Solanaceae with include wide range of cultivars (tomato, tobacco and etc.), 70 aphids species can transmit PVY infection [7]. PVY is distributed almost in all potato production regions: Iran [8], Spain [9], South Africa [10] etc.

virus strain [11], [12]. PLRV/PVY mixed viral infection negatively affect yield depending on the potato variety [13].

Distribution of PVX virus is associated with potato cultivation [14]. PVX is a mechanically transmitted virus [15]. It is considered that harvest losses caused by PVX is the third place after PLRV and PVY and depend on potato cultivars, degree of symptom expression, and environmental conditions etc. [16].

Viral diseases increase during each reproduction because of infected seed material. Also, healthy potato can be infected by several viruses in open filed during a growing season [17].

Production of disease free planting materials has been recognized as the major priority to increase potato production in Georgia. Potato seed imported from abroad are infected in some cases. Absence of certified system of healthy seed production technology in Georgia influenced potato productivity negatively.

The main goal of the research was to study the distribution potato viral infection (PVY, PLRV, PVX, PVM, PVS, and PVA) in Georgian region Akhalkalaki for further selection of virus-free propagated material in Georgia.

II. Materials and Methods

A survey was carried out in the potato growing areas in Georgia. 963 symptomatic and asymptomatic leaves and tubers were collected during summer and early autumn in 2015 and 2016 from Georgian region Akhalkalaki. During the same season adult tubers were collected from 186 samples of ‘Sante’, 204 samples of ‘Laura’, 191 of ‘Jelly’, 208 ‘Red Sonia’, 174 ‘Anushka’. The studied varieties have various country of origin: 'Sante ' is Netherlands variety, ‘Laura” is from Austria, country of origin of ‘Jelly’; ‘Red Sonia’ (originated between the selections designated '546/89 L' and 'E 93/477') and ‘Anushka’ (cross-breeding between ‘Leila’ and “Marabel’) are Germany. All varieties were analyzed by DAS-ELISA as reported by Clark and Adams for virus detection [18].

The 6 leaflets in the 3 directions at the height of 2 m above the ground from each sample were collected. The ELISA test was performed by using extracts from young leaves of the collected samples, also tubers and the serological commercial kits purchased from BIOREBA AG (Switzerland) according to

the manufacturer's Instructions: leaves are homogenized 1:20 (w/v) in extraction buffer (pH 8.2) containing 2% polyvinylpyrrolidone (PVP MW 24,000), 0.02% NaN_3 and 0.05% Tween 20. IgG was diluted in 1000x coating buffer and enzyme-labeled antibody in 1000x conjugate buffer. Yellow color developed after 30-120 min from using pNPP (para-nitrophenyl-phosphate), optical density was measured at 405/ 450 nm on Stat Fax 2100 Microplate Reader (DOTMED®, USA).

The ultimate objective of this research on potato infection is the control of these diseases in the Georgian region of Akhalkalaki. Assessment of potato disease occurrence in Akhalkalaki region of Georgia was performed according to Cooke method using the following formula [19], [20].

$$Diseases\ incidence(DI) = \frac{X}{N}X100$$

where X is number of infected potato and N is total number of potato samples.

$$Disease\ Severity(DS) = \frac{\Sigma(a+b)}{N} + \frac{100}{Z}$$

where Σ (a + b) is the sum of symptomatic potato and their corresponding score scale, N is total number of potato samples and Z is highest score scale. The highest disease rating is five.

III. Results

This research focused on observation spreading of viral diseases in five kinds of potato cultivars: 'Sante', 'Laura', 'Jelly', 'Red Sonia', 'Anushka' from Georgian Region Akhalkalaki in 2015-2016 (Fig. 1).

Fig. 1 Potato Cultivars in Georgian Region Akhalkalaki (Open field)

Potato varieties were tested for 6 types of viruses: PVY, PLRV, PVM, PVX, PVS and PVA. The result showed that viral infections are characterized by different prevalence depending on potato cultivars. Samples were collected according to virus symptoms, but filed symptoms are not always associated with virus infection.

Among all studied potato varieties, the highest infection rate was reported on PVY (21.4%), followed by PLRV (19.7%); the distribution of PVM (3.12%) was minor, but PVS, PVA, PVX did not show any positive reaction with the antibodies. 76 out of 963 tested samples reacted positively to double infection: PVY/PLRV (7.9%) (Table I).

TABLE I
Viral Infected Result of DAS-ELISA Test in Akhalkalaki Region of Georgia

Virus Varieties	Number of tested samples: 963 Infected	Total infected rate (%)
PVY	206	21.4
PLRV	189	19.7
PVM	30	3.12
PVX	-	-
PVS	-	-
PVA	-	-
Mix Infection: PVY/PLRV	76	7.9

The survey of Akhalkalaki region of Georgia showed that most of the potato cultivars were carrying several viral infections. According to the ELISA result, 'Sante' sample contained of PVY (34.64%) and PLRV (31.88%) viruses, whereas the presence of PVM, PVX, PVA and PVS was not detected (Table II).

TABLE II
Result of Virus Testing in Sante

Potato	Virus Varieties	Sample Tested	Positive Simple	
			Number	%
Sante	PVY	254	88	34.64
	PLRV	254	81	31.88
	PVM	254	-	-
	PVX	254	-	-
	PVA	254	-	-
	PVS	254	-	-
Mix infection	PVY/PLRV	254	59	23.22

Survey of potato cultivars: 'Red Sonia' and 'Anushka' showed that in case of 'Sante', PVY infection was characterized highly spreading (15.83% and 35.24%) following PLRV infection (13.94% and 7.78%) whereas distribution of PVM was low (2.86%). PVA, PVX, PVS were not found in cultivars' Anushka' and 'Red Sonia' (Tables III, IV).

It is noted that mix infection (PVY/PLRV) was found in three kind of potato varieties: 'Sante', 'Red Sonia' and 'Anushka' (Tables II-IV).

TABLE III
Result of Virus Testing in Red Sonia

Potato	Virus Varieties	Sample Tested	Positive Simple	
			Number	%
Red Sonia	PVY	208	32	15.38
	PLRV	208	29	13.94
	PVM	208	-	
	PVX	208	-	-
	PVA	208	-	-
	PVS	208	-	-
Mix Infection	PVY/PLRV	208	8	3.84

It has been discovered that PVM was presented only in three types of cultivars: 'Anushka, (2.86%), Jelly (11.57%), and Laura' (6.61%). Except PVM infection, cultivars 'Laura '

and 'Jelly' also were positive to the PLRV virus (Tables V, VI).

TABLE I V
RESULT OF VIRUS TESTING IN ANUSHKA

Potato	Virus Varieties	Sample Tested	Positive Simple	
			Number	%
Anushka	PVY	244	86	35.24
	PLRV	244	19	7.78
	PVM	244	7	2.86
	PVX	244	-	-
	PVA	244	-	-
	PVS	244	-	-
Mix infection	PVY/PLRV	244	9	3.68

TABLE V
RESULT OF VIRUS TESTING IN LAURA

Potato	Virus Varieties	Sample Tested	Positive Simple	
			Number	%
Laura	PVY	136	-	-
	PLRV	136	39	28.6
	PVM	136	9	6.61
	PVX	136	-	-
	PVA	136	-	-
	PVS	136	-	-

TABLE VI
RESULT OF VIRUS TESTING IN JELLY

Potato	Virus Varieties	Sample Tested	Positive Simple	
			Number	%
Jelly	PVY	121	-	-
	PLRV	121	21	17.35
	PVM	121	14	11.57
	PVX	121	-	-
	PVA	121	-	-
	PVS	121	-	-

As the results showed, the highest total infected rate was found in potato cultivars 'Sante' (Table II) and 'Anushka' (Table IV) followed by 'Red Sonia' (Table III), 'Jelly' (Table VI) and 'Laura' (Table VI).

IV. DISCUSSION

Potato viral infection causes considerable losses to the potato industry [21]. Infection becomes permanent and they have a tendency to transmit through vegetative propagation [22]. Using tissue culture technics for producing virus-free plants were reported by different researches [22], [23].

The prevention of viral infection is based on the control of sources of infection; vectors; usage of resistant varieties and etc. [24].

Distribution of viral infection of grapevine and apple varieties is studied in Georgia [25], [26]. Investigation of potato viral infection using DAS-ELISA method enables us to understand real sanitary status of potato diseases. Presence of most harmful PVY and PLRV viruses is considered as a serious problem in Akhalkalaki region of Georgia.

Assessment of spreading all these pathogens using effective laboratory methods will promote the foundation of effective disease monitoring system in Georgia. Assessment of spreading all these pathogens using effective laboratory (Das-Elisa) method is used to avoid the fast and extensive dissemination of infection diseases in potato cultivars 27], [28].

The study represents the extensive survey based on selection of virus-free potato propagated materials for ensuring high-quality crops and management of potato virus spreading in Akhalkalaki region of Georgia. Based on our results of DAS-ELISA immune-assay, distribution of selected viruses among potato varieties was different. Suitable tool for a large-scale disease diagnosis of DAS-ELISA assay allows us to use a single procedure for identification of plant/pathogen combination including symptomless samples.

Intensity of pathogenic impact depends on the potato variety [29]. The research has confirmed that the most harmful virus (PVY) was detected in the collected samples and characterized by relatively high distribution (21.4%) in three types of potato cultivars ('Sante', 'Anushka' and 'Red Sonia'). The virus PLRV showed less spreading evidence (19.7%) than PVY, but it was revealed in all kinds of potato varieties ('Sante', 'Anushka' , 'Red Sonia', 'Jelly' and 'Laura'). The total infected rate of PVM (3.12%) was negligible as compared to others, but PVA, PVX, PVS infection was not revealed.

The results showed a possible relationship between viral infections and potato varieties, resistant ability of potato varieties, transmission pathways of virus, presence of insects and aphids, geographical location from sea level might be related with the different distributions of viral infections among potato cultivars.

Assessment of the real phytosanitary status using Das-ELISA analyses is the most important activity for production of virus free propagated materials of potato cultivars in Georgia.

V. CONCLUSION

The survey of potato viral infection in Akhalkalaki region was conducted for 2 years.

Finally, according to the result, PVY (21.4%) and PLRV (19.7%) viruses showed high distribution whereas the dissemination of PVM virus was low (3.12%) and the presence of PVX, PVA and PVS infections were not revealed. 7.9% of patterns contained double PVY/PLRV infection. Therefore, regular sampling and testing of all stages (lab, greenhouse, open field) in potato seed production system is the main key to avoid reducing yield.

Similar studies enable us monitoring of spreading of viral infection and successfully implementation of certification system for producing virus-free potato seed in Georgia.

ACKNOWLEDGMENT

We acknowledge the Georgian Technical University, Biotechnology Center (Kostava st 77, 0175, Tbilisi, Georgia) and the Ministry of Education, Science, Culture and Sport of Georgia for the financial and technical support for this work.

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