Pakistan Journal of Phytopathology
Published by:
Pakistan Phytopathological Society
www.pakps.com
www.pjp.pakps.com
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Pak. J. Phytopathol.,
Vol. 24 (1): 44-47, 2012.

INCIDENCE OF SIX VIRUSES IN POTATO GROWING AREAS OF PAKISTAN

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ABSTRACT

Incidence of six virus diseases of Potato in Rawalpindi, Islamabad, Faisalabad and Sahiwal were investigated in field samples. Enzyme Linked Immunosorbent Assay (ELISA) tests revealed that Potato virus Y (PVY) was most predominant virus followed by Potato virus X (PVX), Potato virus A (PVA), Potato virus S (PVS), Potato virus M (PVM) and Potato leaf roll virus (PLRV). During the year 2011-12, the average incidence of potato virus diseases in four locations was 52.3%. Single, double, triple, quadruple and quintuple infections were 41.89, 21.78, 16.2, 17.87 and 2.23% respectively. *Chenopodium quinoa* produced local chlorotic lesion against PVX, PVM and PVS. Systemic infection of PVX and no infection of PVM and PVS were observed on *Nicotiana tabacum* cv. Samsun.
Key words: incidence, PVX, PVY, PVA, ELISA.

INTRODUCTION

The cultivated potato (*Solanum tuberosum* L) is the world's leading staple food and vegetable crop and ranked fourth in production after rice, wheat and maize (Rauscher *et al.*, 2006). The potato crop gives 12-15 times more yield per hectare and calories production per unit area is higher then wheat, maize and rice. The potato tuber is an excellent source of carbohydrates, protein and vitamins (MacGillivary, 1953). In Pakistan, potato is cultivated over an area of 127.7 thousand hectares with an annual production of 3726.5 thousand tons (GOP 2011). A significantly high number of pests and pathogens can be carried over from one generation to the next by propagated vegetative material. Among them, at least 37 viruses can naturally infect potato crops. Potato production occurs from three crops during a year; autumn, spring and summer crops in the plains and the hilly areas of Pakistan. High yielding foreign potato varieties significantly increased the yield of potato crop in Pakistan but at the same time resulted new viral problems like PVX, PVY, PVS, PLRV, PVA and PVM which have been reported in spring, summer and autumn potato crop of Pakistan and cause up to 83% yield losses (Mughal and Khalid, 1986). Most viruses can effectively be determined by ELISA tests (Petrrunk *et al*., 1991).

MATERIALS AND METHODS

Sampling: During the Year 2011-12, sampling of potato leaves was conducted in four potato cultivated areas: Rawalpindi, Islamabad, Faisalabad and Sahiwal. A total of 342 samples of 6-8 weeks old field growing potato plants were collected on the basis of virus and viral like symptoms. A single sample was consisted of three single leaflets taken from top, middle and bottom and placed in polythene sample bag. Samples were appropriate labeled to indicate location, sample number and date of collection. These samples were brought to National Agricultural Research Centre (NARC) at Crop Disease Research Program (CDRP) and stored at 4°C in plant virology lab.

Serological detection: All collected samples were tested against PVX, PVY, PVA, PVM, PVS and PLRV in polystyrene microtitre plate through standard Double Antibody Sandwich (DAS) ELISA as described by Clark and Adams (1977) using antibodies (BIOREBA AG kit) and enzyme substrate. ELISA plates were coated with monoclonal Immunoglobulin (IgG) 100µl/well (PVX, PVY, PLRV, PVA, PVM and PVS) diluted at 1:500 in coating buffer and incubated for 3 hours at room temperature followed by washing through 1X phosphate buffer saline tween (PBST) three times at 3 minute intervals. Leaves were chopped into small pieces and ground in sterile pestle and mortal with extraction buffer and sap was filtered through double layer of muslin cloth. Each well loaded 100µl antigen (sap of infected leaf tissue) with micropipette, buffer and healthy samples were also loaded for control and plate was incubated for 24 hours at 4°C. Followed by washing, 100µl of enzyme alkaline phosphatase

(ALKP) conjugated IgG diluted at 1:500 in conjugate buffer was added each well and incubated at room temperature for 3 hours. After washing, substrate buffer (150µl) containing p-nitro phenyl phosphate (1 mg/ml) was added to each well. Incubation was done at room temperature for 55 minutes in dark and the reaction was observed visually by yellow colour and also read in ELISA reader (EPSON LX-300) at 405 nm. A sample is considered as virus infected when the absorbance of 405 nm was at least thrice of that healthy control. Incidence of potato viruses was calculated by following formula

$$\% \text{ Incidence of PVA or PVM or PVS or PLRV or PVX or PVY} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Host range studies: ELISA confirmed samples were selected and further evaluated for host range studies. Experimental host plant species *Chenopodium quinoa* and *Nicotiana tabacum* cv. Samsun were raised in insect free glass house. The potting mixture consisted of sterilized field soil, sand and farm yard manure (pH 7.2). The experimental host plants were dusted with a fine covering of carborandom 500 by using a throat spray. ELISA highly positive samples were ground in inoculation buffer. A finger was dipped in the sap and gently rubbed on leaves of plants. Plants were inoculated with a carborandom dust alone with inoculated buffer sap for control. Inoculated leaves surface was washed with cold tap water. Plants were kept in green house with 12 hours light period, a temperature of 25 ± 5°C and 50 to 70% relative humidity and observed 1 to 3 weeks after inoculation.

RESULT AND DISCUSSION

Symptomlogy: Stunting, systemic vein clearing, mosaic, mottling, curling, shortening of leaves, dark green and vein-banding were observed on of the potato. In few plants with leaf roll symptoms, entire leaflets were rolled while in others plants, it was only restricted to the leaflet bases. Symptomlogy is not a reliable method for confirmation of viruses but it is an initial step to disease diagnosis because symptom development is due to many factors such as insect sucking, environmental conditions, nutrition deficiency, growth stage, time of infection, host genotype, virus strain, etc (Batool *et al*., 2011). For further confirmation, these samples were preceded for serological detection.

Serological detection: PVY was most predominant in all locations and other remaining viruses varied in their order of frequency (Table 1).

Table 1: Out of 342 only 179 samples showed their reaction against DAS-ELISA.

virus tested	Single	Double	Triple	Quadruple	Quintuple
PVX	19				
PVY	37				
PLRV	1				
PVA	7				
PVM	7				
PVS	4				
PVX+PVA		1			
PVX+PVY		20			
PVX+PVM		1			
PVX+PVS		1			
PVY+PVA		5			
PVY+PVM		2			
PVY+PVS		6			
PVY+PLRV		1			
PVA+PVS		1			
PVM+PVS		1			
PVX+PV+PVS			5		
PVX+PVA+PVM			1		

PVX+PVY+PLRV	1			
PVX+PVY+PVS	3			
PVX+PLRV+PVS	6			
PVX+PVY+ PVA	1			
PVX+PVM+PVS	2			
PVY+ PVA+PVS	4			
PLRV+PVA+PVM	1			
PVA+PVM+PVS	5			
PVX+PVY+PLRV+PVS		10		
PVX+PVY+PVM+PVS		2		
PVX+PVY+PVA+PVS		3		
PVX+PVA+PVM+PVS		16		
PVY +PVA+PVM+PVS		1		
PVX+PVY+PLRV+PVM+PVS			1	
PVX+PLRV+PVA+PVM+PVS			1	
PVX+PVY+PVA+PVM+PVS			2	

PVX, PVY, PVA, PVM, PVS and PLRV represent Potato viruses X, Y, A, M, S and potato leaf roll virus respectively.

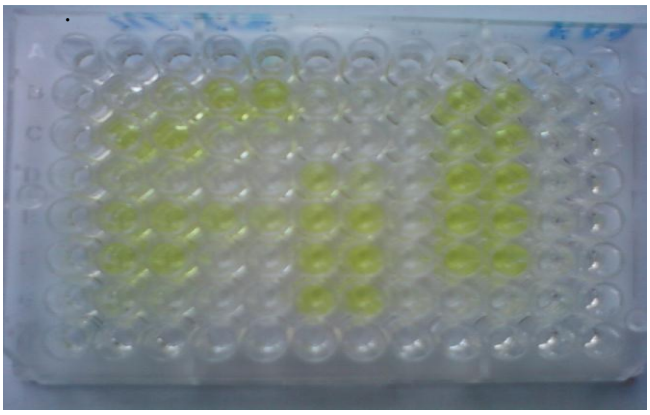

Fig.1. Reaction of PVA, PVM, PVS, PLRV, PVX and PVY in ELISA plate.

Out of 342 samples only179 samples were confirmed through DAS-ELISA with one or more viruses.

Table 2: Reaction of indicator host plant species to potato viruses.

Indicator host plants	PVX	PVM	PVS
Chenopodium quinoa	Cll	Cll	Cll
Nicotiana tabacum cv. Samsun	SI	NI	NI

ELISA is used for confirmation of viruses in experimental host plants. Cll= chlorotic local lesion, SI = Systemic, NI= no infection

Yellow colour indicates that there was positive reaction with virus antigen using monoclonal antibodies of PVA, PVM, PVS, PLRV, PVX and PVY (Fig1). The relative frequency of infection by PVY, PVX, PVA, PVS, PVM and PLRV was 55.3, 53.6, 40.7, 40.2, 24 and11.7% of infected samples respectively. Single, double, triple, quadruple and quintuple infections were 41.89, 21.78, 16.2, 17.87 and 2.23% respectively. ELISA tests are more reliable for detection and identification of viruses. This is the reason that ELISA confirmed the potato viruses only in 179 field samples. Infection of potato viruses in all location with different percentage is similar as reported by Mughal and Khalid (1986). Burhan *et al.* (2006) also reported prevalence of PVX and PVY in Faisalabad

Fig 2: chlorotic local lesion of *Chenopodium quinoa* against potato viruses.

Host range: Potato viruses are also transmitted by mechanical contamination during cultural practices. PVX, PVM and PVS produced local coloristic lesion on *C. quinoa* (Fig 2), no reaction of PVM and PVS while systemic infection of PVX was observed on *N. tabacum cv.* Samsun (Table 2). The same host range study was reported by Pourrahim *et al*. (2007).

This study seems to indicate that low yield of potato in these potato growing areas may be due to high infection of potato viruses (52.3%). High incidence of PVY need for further investigations into different strains of PVY that may occur in Pakistan. Available high yielding commercial varieties or advance lines in Pakistan has not shown durable resistance against viruses (Qamar *et al*., 2003). This might be due to the continuous introduction of viruses through imported seeds, occurrence of the carrier or vector of and chemicals are not available for directly controlling viral diseases of plants in the field (Ahmad and Ahmad, 1995). Thermotherapy gave 46% reduction in virus concentration and Nascimento *et al*. (2003) achieved elimination of PVY after treatment of plantlets with high temperatures. Resistant or tolerant potato varieties against viruses, meristem culture, treatment with chemotherapy and thermotherapy, insect pests along with weeds control may play a vital role in reducing yield losses in potato crop. Molecular identification of these viruses will also be helpful in developing an integrated control strategy and resistance source against potato virus diseases and thus improve the farmer's income.

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