Screening of potato germplasm against RNA viruses and their identification through ELISA

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ABSTRACT: Fifteen potato advance lines were screened against Potato Leaf Roll Virus (PLRV), Potato Virus X (PVX), Potato Virus Y (PVY) under favorable natural field conditions and in vitro by using double antibody sandwich ELISA (DAS-ELISA) against six potato viruses viz; PLRV, PVX, PVY, Potato Virus S (PVS), Potato Virus A (PVA) and Potato Virus M (PVM) at Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan during 2012-2013. On the basis of symptomatology, only two lines FD70-1 (PRI RED), SL15-26 were found free of symptoms to PLRV. No one genotype was found to be highly resistant against PVX and PVY. Environmental factors had a great influence on disease severity. In ELISA screening no one advance line was highly resistance against six potato viruses. Three lines namely viz; FD70-1 (PRI RED), SL15-24 and SL15-26 were found resistant, five moderately susceptible and seven susceptible in case of PLRV. In PVY, two advance lines i.e. FD70-1 (PRI RED) and SL14-05 were found resistant. In PVX, only one line SL15-26 was found to be resistant. In PVA, four resistant advance lines, eight moderately susceptible and three were found as susceptible. In PVS, only one resistant genotype, six moderately susceptible and eight were found as susceptible. Likewise in PVM there is no genotype as a resistant, eight moderately susceptible and seven were found as susceptible. The resistance germplasm of potato screened under field conditions and by ELISA of different potato viruses may be used to produce the virus free germplasm in the next breeding programme.

Key Words: Potato viruses, *Solanum tuberosum* L, Symptomatology, ELISA, correlation

INTRODUCTION: Potato (*Solanum tuberosum* L,) belongs to the family Solanaceae and important vegetable crop in the world. It is a rich source of protein, starch, vitamins (B & C) and minerals i.e. 2% protein, 18% starch, 1% vitamins, minerals and water is about 69% herbaceous plant.

In the world, it is grown in 140 countries (Haase, 2008). In Pakistan, the cultivated area of potato has been recorded 161.9 thousand ha with production of 3.5071 million tons. (Anonymous. 2014).There are many plant pathogens attack on potato and overcome the potato production in Pakistan in all seasons, i.e. autumn, spring and summer in hilly and plain areas. Large numbers of viruses remain as a host on potato species. There are 37 viruses which naturally infect the potato species. (Beemster and DeBokx, 1987; Salazar 1996; Jeffries, 1998). There are many viruses of potato which occur world widely i.e. potato leaf roll (PLRV), potato virus Y (PVY), potato virus X (PVX), potato virus A (PVA), potato virus S (PVS), potato virus M (PVM), potato virus V (PVV), potato mop top virus (PMTV) and potato aucuba mosaic virus (PAMV) and the other are ecologically important (Brunt 2001, Mughal, 1990). About 1 to 17 % in 1st year & 37 to 56 % in subsequent year's deterioration has been reported due to viruses (Jagidar *et al*., 1982). In Pakistan the losses has been reported approximately 50% (Mughal and Khalid, 1985) and up to 83% due to viruses (Ahmad *et al*., 2011a) whilst yield losses caused by PLRV and PVY up to 70%, PVX 10 to 25%, PVA 40%, PVS 20%, and PVM 30% were recorded (Mughal *et al*., 1988). Serological procedures are the most reliable method for detection of plant viruses form the most reliable method for detection and quantitative assay of viruses. In this study, for the detection of viruses the direct double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) is used (Clark and Adams 1977). In the present study we have studied the presence or absence of the six potato viruses including Potato Leaf Roll Virus (PLRV), Potato Virus Y (PVY), Potato Virus X (PVX), Potato Virus A (PVA), Potato Virus S (PVS) and Potato Virus M (PVM) on fifteen advance lines/varieties using DAS-ELISA. The objective of the study was to find the resistance source against Potato viruses.

MATERIALS AND METHODS: For screening purpose, 15 potato advance lines/advanced lines were planted during the year 2012-13 in the research area of Department of Plant Pathology in three replications. All advance lines of potato were selected randomly for each replication for screening against PLRV, PVX and PVY. The distance between P×P was 30cm and R×R was 60cm. Observations were initiated from 15 of December, 2012 to 28 February, 2013. Potato varieties were classified for resistance against PLRV, PVX and PVY on the basis of standard scale as given in Table 1 (Mughal and Khan, 2001). In order to record the incidence of PLRV, PVX and PVY following formula was used: % Incidence of PLRV, PVX or PVY =

$$\frac{\text{No. of infected plants/ unit area}}{\text{Total no. of plants/ unit area}} \times 10$$

To check the significant influence on disease severity environmental data consisting of maximum temperature, minimum temperature, rainfall, and relative humidity were subjected to correlation and regression analysis. Confirmation of PLRV, PVY, PVX, PVA, PVS, and PVM through Serological Test (ELISA) buffer solutions required for DAS ELISA were prepared by following materials and methods. The procedure for double antibody sandwich ELISA (DAS-ELISA) involved the following steps:

Step 1

- Dissolved 20ul antibody of six viruses in 20 ml antibody coating buffer separately for each virus and made the desired solution.
- Coated the solution @ 200ul for each well in the microtitre plate with the help of micropipette.
- Then incubated the microtitre plate at 4^{o} C overnight

Step 2

Leaf samples of different advance lines were collected for testing through ELISA against

PLRV, PVY, PVX, PVS, PVA and PVM as described by Clark and Adams (1977).

- After incubation the plates were washed with washing buffer 3 times.
- The collected leaf samples mixed with virus extraction buffer @ 1:10 with the help of mortar and pestle to extract the sap and homogenized. The six different plates for six viruses were filled with the sap @ 200ul for each well with the help of micropipette.
- Before adding the samples into the plates, filled 2 wells for the positive control and 2 for the negative control of the six viruses separately.
- The coated plate was again incubated for overnight at 4^{o} C.

Step 3

- Washed the plates with washing buffer 3times and then take conjugate 20ul and added conjugate buffer 20ml separately for each virus.
- Poured the plates at the rate of 200ul for each well.
- Incubated the plate at 4^{o} C overnight.

Step 4

- Washed the plate 3 times with washing buffer and then take 20ml substrate buffer and 1 PNP tablet.
- Mixed tablet in substate buffer.
- Poured the plates @ 200ul for each well with the help of micropipette.
- Put the plate for 30 minutes at room temperature and reaction was visually observed for the development of yellow color.
- The reaction was stopped by adding 50µl 3M NaOH to each well.
- The results were compiled by the following scale
  - Deep Yellow= strong (+++)= Susceptible
  - Moderate Yellow = Moderate(++) = Moderately Susceptible
  - No color = Free = Resistant

Table 1. Disease Rating Scale for PLRV, PVX and PVY

Index	Reaction	PLRV	PVX	PVY
0	Highly Resistance	No visible symptoms.	No visible symptoms.	No symptoms
1	Resistance	Rolling of leaves in case of primary infection and lower leaves in case of secondary infection, erect growth	Mild mottling on the upper leaves.	Blackening and banding of vein on few leaves. Mosaic starting on all leaves.
2	Moderately Resistance	Rolling of leaves extending, leaves become stiff and leathery, stunting of plants and erect growth	Inter venial mosaic symptoms on more than one leaf.	Blackening and banding of vein on all leaves. Narrowing of leaves. Venial necrosis, severe mosaic, Leaf crinkling.
3	Moderately Susceptible	Short internodes, papery sound of leathery leaves, rolling and stunting of whole plants. Young buds are slightly yellowish and purplish	Mosaic symptoms on all leaves.	Rugosity and leaf drop streak, dwarfing
4	Susceptible	Clear rolling of leaves, severe stunting, few tubers and tuber necrosis	Distinct mosaic symptoms on all leaves.	Lower leaves dead, drooping collapse of plants with very small tubers.
5	Highly Susceptible	All above symptoms and small number of small sized tubers.	All above symptoms and small number of small sized tubers	All leaves dead, stem dead or drying .

RESULTS AND DISCUSSION: Screening against PLRV: The screening experiment conducted under field conditions showed that out of fifteen advance lines, two advance lines were found highly resistant against PLRV i.e. FSD-69-1 and SL-15-26; three advance lines SL-15-24, SL-14-05 and SL-14-13 showed resistant response; two advance lines SL-13-39 and FD-74-28 exhibited moderately resistant response; two advance lines i.e. SL-13-02 and SL-14-37 showed moderately susceptibility; four advance lines FSD-RED, SL-15-21, SL-13-64 and SL-13-33 were susceptible and two advance linesFD-73-38 and SL-15-04 exhibited highly susceptible response against PLRV (Table 1). Batool et al, (2011) also screened 29 potato varieties/lines against PLRV under natural field conditions and concluded that only one line i.e., 394032-16 was found free of symptoms while SH-5, FD 1-8 and FD 1-9 were considered as moderately resistant; 19 genotypes were considered as moderately susceptible and six were susceptible to PLRV.

Screening against PVY: Out of fifteen advance lines there were no genotype found to be highly resistant against PVY. There were found two advance lines FSD-69-1 and SL-14-05 but four SL-13-64, SL-14-13, SL-14-37 and SL-15-21 were moderately resistant, Susceptibility factor also appeared there, Five advance lines such as FSD-RED, FD-74-28, FD-73-38, SL-15-26 and SL-15-04 were found moderately susceptible, SL-15-24, SL-13-33 and SL-13-02 these were found as susceptible advance lines and there was only one genotype i.e. SL-13-39 which showed high susceptibility (Table 2). Ahmad *et al*, (2011a) also screened potato varieties/lines consisting of 28 against the PVY under field conditions in two consecutive seasons 2008-09 and 2009-10. He reported that out of 28 genotypes five genotypes FD3713, OCEANIA, FD13, FD32-2 and TPS-9801 were found to be resistant (R) against PVY and four genotypes, Arterix, FD3-10, Orla and Fsd-Red were susceptible (S). Four genotypes were found moderately resistant (MR), nine genotypes were found moderately susceptible (MS) against PVY

Screening against PVX: Out of fifteen advance lines there were no genotype found to be highly resistant against PVX. There were found three advance lines FSD-69-1, SL-15-26 and SL-14-13resistant, no highly resistant genotype but three SL-13-33, SL-14-05 and SL-15-24 were moderately resistant, According to disease rating scale there were found susceptible advance lines also, like SL-15-21 and SL-15-04 were moderately resistant, and three advance lines such as FD-74-28, FD-73-38 and SL-13-39 were found susceptible and three advance lines which showed highly susceptibility i.e. SL-13-02, Sl-14-37, and FSD-RED (Table 3). Ahmad *et al*, (2011a) also screened potato germplasm under field conditions that was consisted of twenty eight genotypes against the PVX during two consecutive years in 2008-09 and 2009-10. He reported that three genotypes FD3-9, 393574-61 and FD3-10 found to be highly resistant (R) against PVX. Only three genotypes Arterix, Mirrato and Desiree were moderately susceptible (MS) while eight genotypes were moderately resistant (MR) and six were found to be resistant (R) against PVX.

Correlation with environmental factors: Correlation of weekly maximum and minimum air temperature, relative humidity and rain fall with PLRV, PVY and PVX disease severity was determined at genotype/variety level. In PLRV, there was a positive correlation with maximum temperature, minimum temperature, rainfall and negatively correlated with relative humidity in following advance lines i.e. SL13-64, SL13-02, SL15-04 and SL13-39 (Figure 1-4). Similarly in case of PVY there is also a

Table 2: Progression of PLRV in potato varieties in disease screening nursery

S. No.	Advance lines	PLRV		PVX		PVY	
		Disease severity Index	Level of Resistance /Susceptibility	Disease severity Index	Level of Resistance /Susceptibility	Disease severity Index	Level of Resistance /Susceptibility
1	FD70-1 (PRI-RED)	0	HR	1	R	1	R
2	FSD RED	4	S	3	MS	5	HS
3	SL15-21	4	S	2	MR	3	MS
4	FD74-28	2	MR	3	MS	4	S
5	FD73-38	5	HS	3	MS	4	S
6	SL15-24	1	R	4	S	2	MR
7	SL13-64	4	S	2	MR	5	HS
8	SL15-26	0	HR	3	MS	1	R
9	SL14-05	1	R	1	R	2	MR
10	SL14-13	1	R	2	MR	1	R
11	SL13-33	4	S	4	S	2	MR
12	SL13-02	3	MS	4	S	5	HS
13	SL14-37	3	MS	2	MR	5	HS
14	SL15-04	5	HS	3	MS	3	MS
15	SL13-39	2	MR	5	HS	4	S

Table 3. Results of Enzyme linked Immunosorbent Assay (ELISA) of six potato viruses during 2012-2013

Sr. no.	Advance lines	PLRV		PVX		PVY		PVA		PVS		PVM	
		ELISA results	Resistance/ Susceptibility	ELISA results	Resistance/ Susceptibility	ELISA results	Resistance/ Susceptibility	ELISA results	Resistance/ Susceptibility	ELISA results	Resistance/ Susceptibility	ELISA results	Resistance/ Susceptibility
1	FSD70-1 (PRI RED)	-	R	++	MS	-	R	-	R	-	R	++	MS
2	FSD RED	+++	S	+++	S	++	MS	+++	S	++	MS	+++	S
3	SL15-21	+++	S	++	MS	++	MS	++	MS	+++	S	+++	S
4	FD74-28	++	MS	+++	S	+++	S	++	MS	++	MS	++	MS
5	FD73-38	+++	S	+++	S	++	MS	-	R	+++	S	+++	S
6	SL15-24	-	R	++	MS	+++	S	++	MS	++	MS	++	MS
7	SL13-64	+++	S	+++	S	++	MS	++	MS	+++	S	+++	S
8	SL15-26	-	R	-	R	+++	S	+++	S	++	MS	++	MS
9	SL14-05	++	MS	++	MS	-	R	-	R	++	MS	++	MS
10	SL14-13	++	MS	-	R	++	MS	++	MS	+++	S	+++	S
11	SL13-33	+++	S	++	MS	+++	S	+++	S	+++	S	+++	S
12	SL13-02	++	MS	+++	S	+++	S	++	MS	+++	S	++	MS
13	SL14-37	+++	S	+++	S	++	MS	++	MS	++	MS	++	MS
14	SL15-04	+++	S	++	MS	+++	S	-	R	+++	S	+++	S
15	SL13-39	++	MS	+++	S	+++	S	++	MS	++	S	++	MS

positive correlation between maximum temperature, minimum temperature, rainfall and negative correlation with relative humidity in FD73-38, SL15-24, SL15-26 and SL13-39 advance lines (Figure 5-8). Likewise there are four advance lines SL15-21, FD74-28, FD73-38 and SL13-02 had a positive correlation with maximum temperature, minimum temperature, rainfall and negative correlation with relative humidity (Figure 9-12). Ahmad *et al*, (2011b) also conducted correlation studies between environmental factors and PVX and PVY infection in selected genotypes of potato. Correlation studies revealed that negative and significant interactions in case of maximum and minimum temperature were noted but in case of relative humidity and rainfall the interaction was positive and significant was seen.

ELISA Test result: ELISA test was made for the confirmation of potato viruses from the samples collected from field is subjected to ELISA. A positive reaction used as antigen agent with PLRV, PVY, PVX, PVA, PVS and PVM monoclonal antibodies, whereas healthy tissues gave negative reaction and these results clearly revealed that PLRV, PVY, PVX, PVA, PVS and PVM were present in the field samples. Development of yellow color in the wells indicated the presence of a virus and its intensity which was proportional to the concentration of virus in the plant. Therefore, the positive and negative samples were sorted out by visual observation of yellow color. Although symptom expression was indicating the incidence of PLRV, PVY, PVX, PVA, PVS and PVM in the field samples but ELISA test confirmed the virus in the samples. Out of 15 genotypes, only one genotype FD69-1 exhibited resistance response against four potato viruses PLRV, PVY, PVS and PVA while it was moderately susceptible against PVX and PVM. Genotype FD73-38 was resistance against PVA; SL15-24 was resistant against PLRV; SL15-26 showed resistance against PLRV and PVX; SL14-05 was resistance against PVY and PVA while genotype SL14-13 showed resistance against PVX. Muhammad *et al*. (2013) also determined the reaction of 20 potato genotypes using DAS- ELISA against these six potato viruses and reported the resistance cultivars and concluded that resistant cultivars may be useful for the development of potato cultivars resistant to potato viruses. Schneller *et al*. (2003) also reported PVA in Nyand ME during 2002-2003 growing season. Leaf samples of 12 plants were collected in research and commercial potato plots and tested by ELISA. It is estimated that 83 % of the samples showed the reaction. Zhang (2004) also studied more than 2000 samples of potato infected by viruses, tissue culture potato and mini potato tubers were deducted and identified by TAS-ELISA which was collected from main potato growing areas of Yunnan. PVX, PVY, PLRV, PMTV and PVM were detected and potato yield reduced evidently along the raising virus infection rate. Clark and Adam (1977) also observed similarly positive reaction with PVA infected tissues. The color reaction was moderate yellow to dark yellow. All the varieties and lines were subjected to double antibody sandwich ELISA (DAS- ELISA), using monoclonal antibodies. Ahmad *et al*, (2011a) also identified resistant sources of potato through serological ELISA test against PVX and PVY to control these viruses is an economical way.

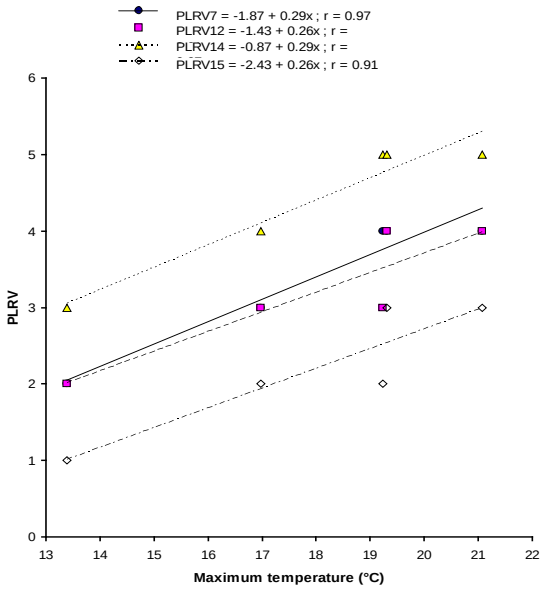

Figure 1. Relationship between relative humidity and PLRV (2012-13) for varieties PLRV7= SL-13-64, PLRV12=SL-13-02, PLRV14= SL-15-04 and PLRV15=SL-13-39

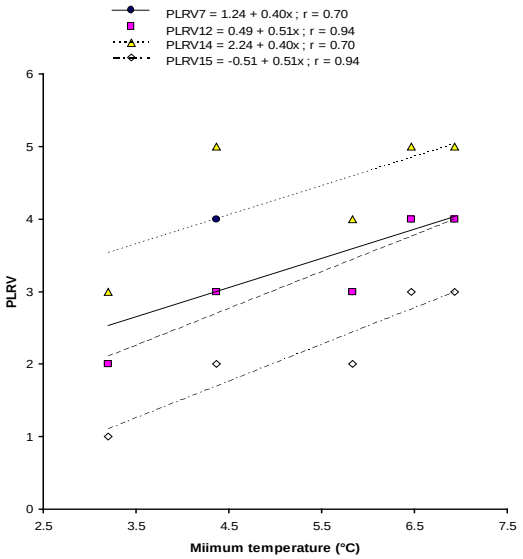

Figure 2. Relationship between minimum temperature and PLRV (2012-13) for varieties PLRV7= SL-13-64, PLRV12=SL-13-02, PLRV14= SL-15-04 and PLRV15=SL-13-39

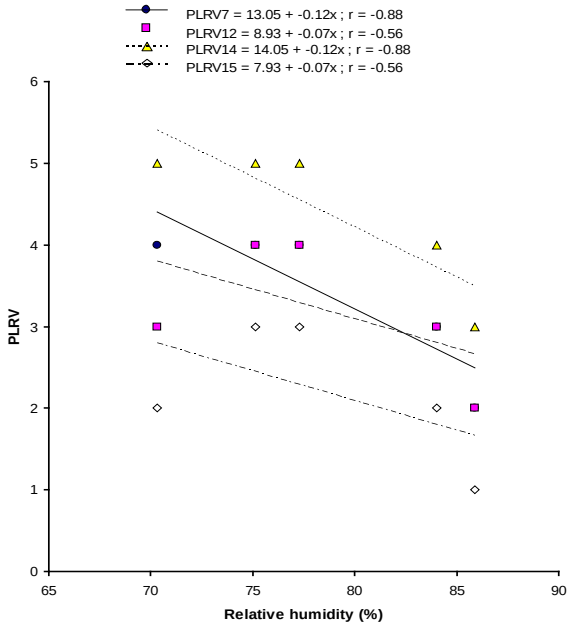

Figure 3. Relationship between relative humidity and PLRV (2012-13) for varieties PLRV7= SL-13-64, PLRV12=SL-13-02, PLRV14= SL-15-04 and PLRV15=SL-13-39

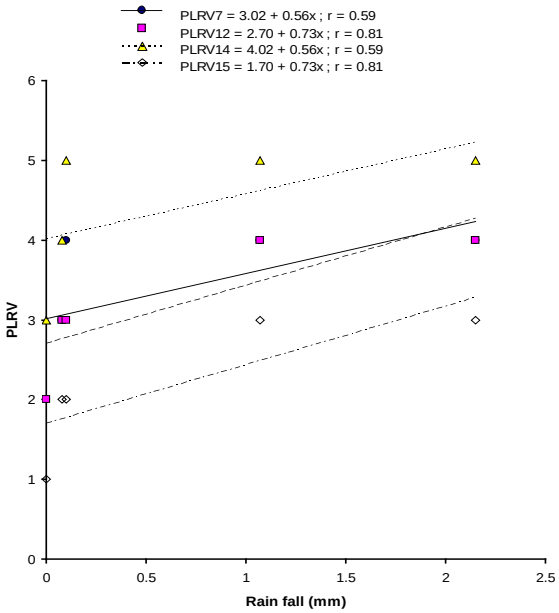

Figure 4. Relationship between relative humidity and PLRV (2012-13) for varieties PLRV7= SL-13-64, PLRV12=SL-13-02, PLRV14= SL-15-04 and PLRV15=SL-13-39

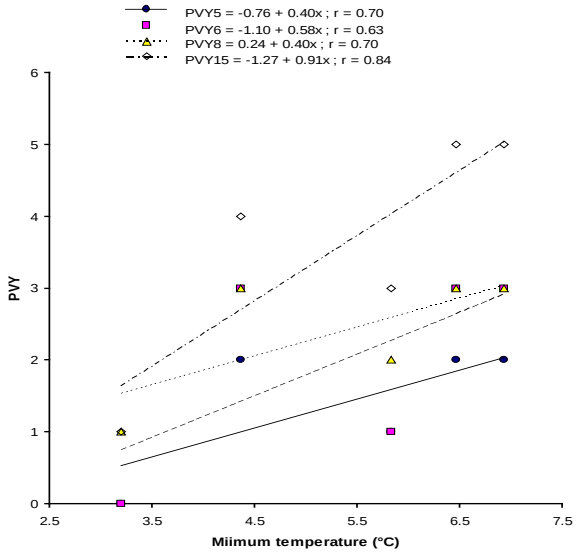

Figure 5. Relationship between maximum temperature and PVY (2012-13) for varieties PVY5= FD-73-38, PVY6=SL-15-24, PVY8= SL-15-26 and PVY15=SL-13-39

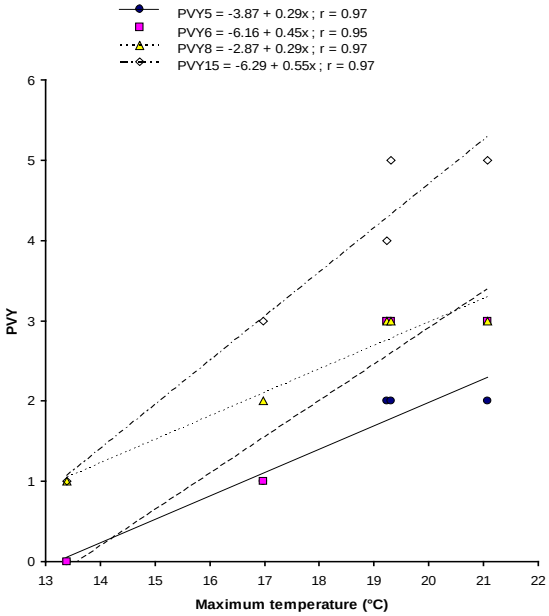

Figure 6. Relationship between minimum temperature and PVY (2012-13) for varieties PVY5= FD-73-38, PVY6=SL-15-24, PVY8= SL-15-26 and PVY15=SL-13-39

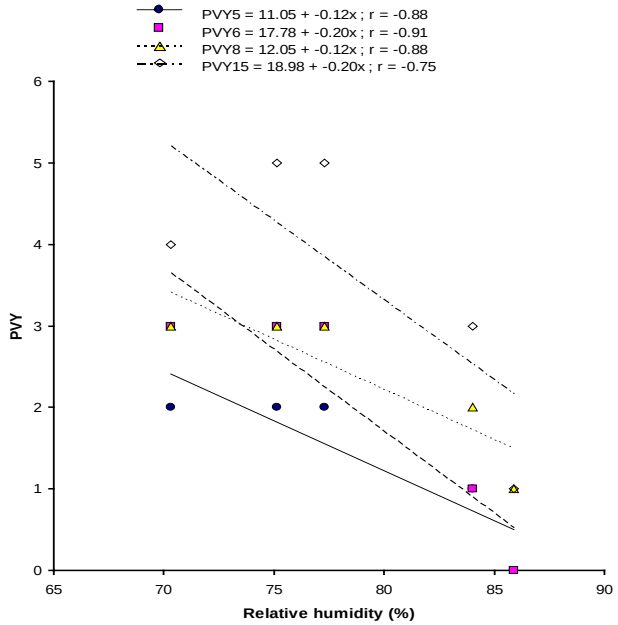

Figure 7. Relationship between relative humidity and PVY (2012-13) for varieties PVY5= FD-73-38, PVY6=SL-15-24, PVY8= SL-15-26 and PVY15=SL-13-39

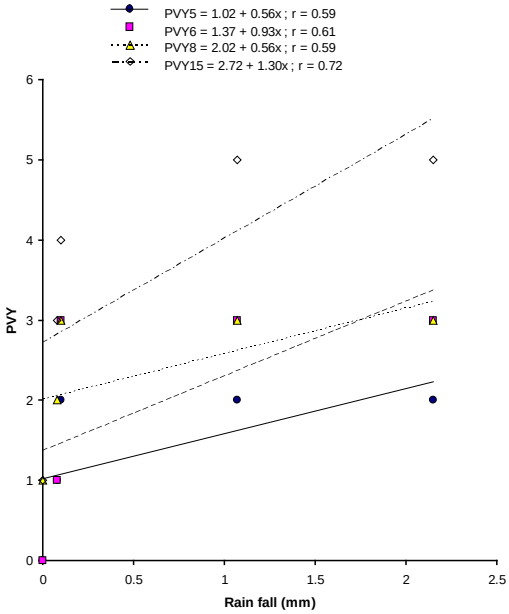

Figure 8. Relationship between Rain fall and PVY (2012-13) for varieties PVY5= FD-73-38, PVY6=SL-15-24, PVY8= SL-15-26 and PVY15=SL-13-39

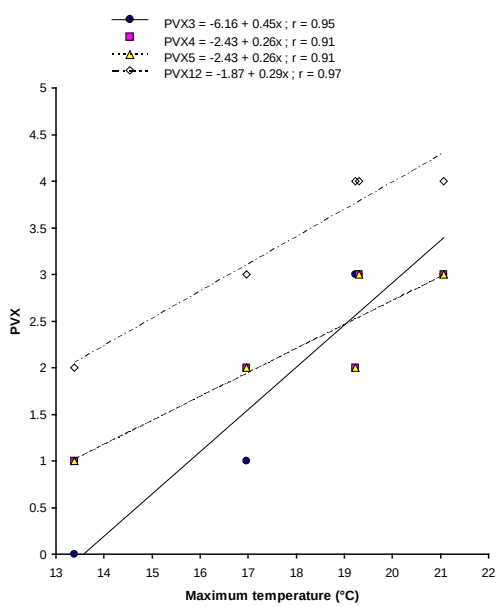

Figure 9. Relationship between maximum temperature and PVX (2012-13) for varieties PVX3 =SL-15-21, PVX4 =FD-74-28, PVX5=FD-73-38, and PVX12=SL-13-02

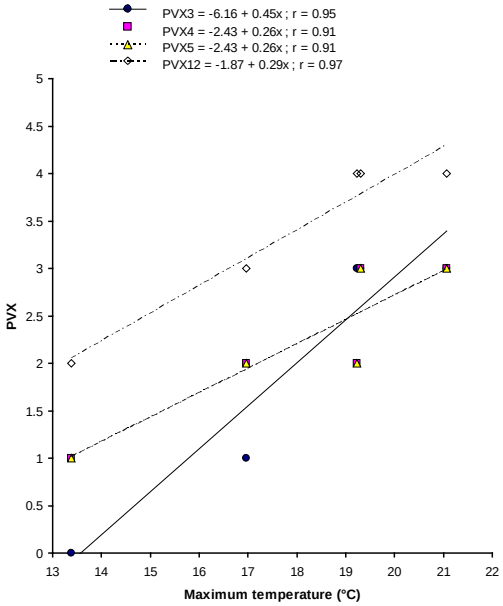

Figure 10. Relationship between minimum temperature and PVX (2012-13) for varieties PVX3 =SL-15-21, PVX4 =FD-74-28, PVX5=FD-73-38, and PVX12=SL-13-02

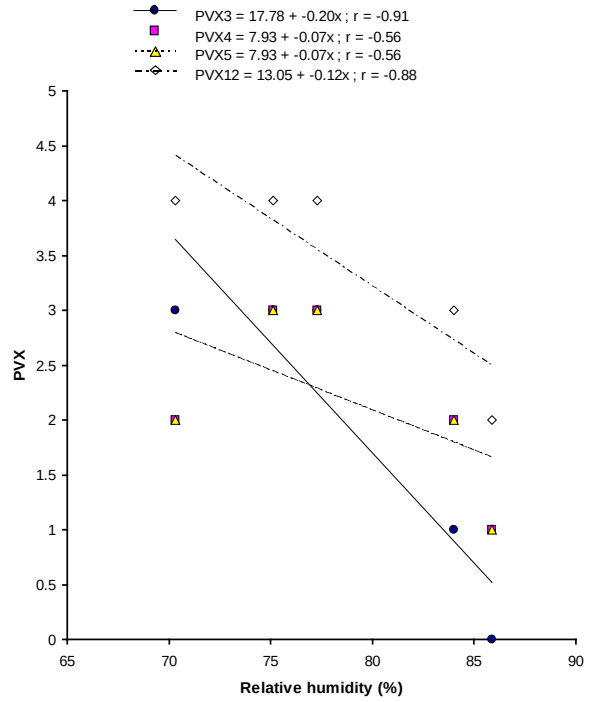

Figure 11. Relationship between relative humidity and PVX (2012-13) for varieties PVX3 =SL-15-21, PVX4 =FD-74-28, PVX5=FD-73-38, and PVX12=SL-13-02

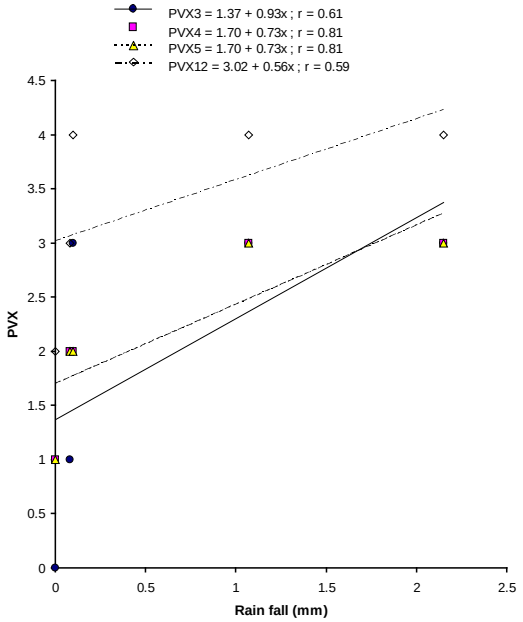

Figure 12. Relationship between Rain fall and PVX (2012-13) for varieties PVX3 =SL-15-21, PVX4 =FD-74-28, PVX5=FD-73-38, and PVX12=SL-13-02

CONCLUSION: PLRV, PVX and PVY are important, serious, widespread and destructive viruses in Pakistan. Same as PVA, PVS and PVM also spread but in field condition it is difficult to screen out these viruses. Different potato advanced lines were screened against different potato viruses such as PLRV, PVX and PVY under natural field conditions and by using serological technique ELISA to find resistance source against six viruses of Potato including PLRV, PVX, PVY, PVA, PVS and PVM. Effect of environmental condition on symptoms expression and development was also investigated. It was found that the potato advance lines varied greatly in their resistance and susceptibility to PLRV, PVX and PVY. In the screening experiment, Out of 15 advance lines, FD70-1 found highly resistant against PLRV, PVY and PVX. Advance line SL15-26 and SL14-13 found resistant against PLRV and PVX and important point is that none of the advance line was found resistant against PVM. Regression analysis revealed that increase in temperature and rainfall supports disease severity while in case of relative humidity it decreases. Advance line FD70-1 is resistant against PLRV, PVY, PVA and PVS in serological assay. Advance line SL15-26 found resistant against PLRV and PVX. Advance lines FD73-38, SL14-05 and SL15-04 also found resistant against PVA. By this study it has been shown that FD69-1 FD70-1 were resistant in many cases similarly SL15-26 was found to be resistant against different viruses so these should be used in further breeding process.

LITERATURE CITED

Ahmad N, Khan MA, Khan NA, Binyamin R, Khan MZ (2011a) Identification of resistance source in potato germplasm against PVX and PVY. Pak J Bot 43: 2745-2749.

Ahmad N, Khan MA, Ali S, Khan NA, Binyamin R, Sandhu AF, Rehman A (2011b). Epidemiological studies and management of potato germplasm against PVX and PVY. Pak J Phytopathol 23: 159-165.

Anonymous (2014) Pakistan Economic Survey. Govt. of Pakistan, Finance Division, Economic Advisor's Wing, Islamabad p:30.

Batool A, Khan MA, Farooq J, Mughal SM, Iftikhar Y (2011) Elisa-based screening of potato germplasm against potato leaf roll virus. J Agric Res 49: 57-63.

Beemster ABR, de Bokx JA (1987) Survey of properties and symptoms. In: de Bokx, 84-113. Pudoc, Wageningen, The Netherlands.

Brunt AA (2001) The Main Viruses Infecting Potato Crops. *In*: Virus and Virus-like Diseases of Potatoes and Production of Seed-Potatoes (Ed. G. Loebenstein *et al*.), pp. 65-67, Kluwer Academic Publishers, The Netherlands.

Clark MF, Adams AN (1977) Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. Gen Virol 34: 475-483

Haase NU (2008) The canon of potato science: 50. The nutritional value of potatoes. Potato JA and van der Want JPH (eds) Viruses of Potatoes and Seed Potato Production

Jagidar AP, Jandan MD, Solangi BA (1982). Studies on the rate of degeneration of some potato varieties in Sindh. Pak J Agri Res 2: 115-118.

Jeffries CJ (1998) Potato. FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm, No. 19, 177 pp. FAO & IPGRI, Rome.

Mughal SM (1990) Virus and virus like diseases of potato in Pakistan.

FAO/DANIDA training course. Proceeding (Jan. 10-21, 1998) FSC &RD, Islamabad. P 166-175. *In*: Quality Seed Potato Production in Northern Areas 2003. FSC & RD. MINFAL. G. O. Pak. Islamabad P: 38-40.

Mughal SM, Khalid S (1985) Virus diseases in relation to potato production in Pakistan, Proc. Pak-Swiss Potato Dev. Project Islamabad 157-165.

Mughal SM, Khalid S, Gillani TS, Devoux A (1988). Detection of potato viruses in Pakistan. Proc. 2nd Triennial conf., Jim. 12-26, Kuming, China p. 189-190.

Muhammad S, Shahbaz M, Javed MT, Iqbal M, Tariq MS, Shahid MTH, Khan NH (2013) ELISA based screening of potato varieties / lines against potato viruses. Int J Biol Biotech 10: 645-650.

Salazar LF (1996). Potato Viruses and their Control, 214 pp. Intern. Potato Center, Lima, Peru.

Schneller A, Paya R, Perry KL, Gary SM (2003). Potato virus survey in New York and Maine and characterization of PVY isolates. Am J Potato Res 156-159.

Zhang Z (2004). Potato viruses and detection system of virus free potato seedling in Yunnan province, China. Biotechnology research institute, Yunnan 156-159pp.