Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

RECOMBINANT POTATO VIRUS Y, A SOARING THREAT FOR POTATO SEED PRODUCTION AND REACTION OF EXISTING POTATO GERMPLASM TO EMERGING RECOMBINANT STRAINS

[a]Muhammad Ussama-Yasin*, [a]Muhammad Burhan, [a]Azhar Mustafa, [a]Shaukat Ali, [b]Salman Ghuffar, [c]Waseem Abbas, [a]Muhammad Z. Niaz, [d]Tariq Javaid,

[a] *Plant Pathology Research Institute, Faisalabad, Pakistan.*
[b] *Vegetable Research Station, Sahiwal, Pakistan.*
[c] *Vegetable Research Institute, Faisalabd, Pakistan*.
[d] *Potato Research Institute, Sahiwal, Pakistan.*

A B S T R A C T

Potato Virus Y has become the most important virus of Potato crop. Various strains of Potato Virus Y has emerged which are affecting the yield of crop and causing economic losses. Due to a conducive climatic conditions for aphid vector the only viable method for control of PVy is identification and cultivation of resistant varieties. Three year's trial was conducted to identify advance lines and varieties showing resistance towards prevalent strains of Potato Virus Y. PVY^{O}, PVY^{LD} and PVY^{VN} were found most virulent and destructive. No line was found resistant, 3 lines showed moderately resistant response, 5 lines were moderately susceptible, 12 lines was susceptible whiles 2 lines shown highly susceptible response. Incidence and Severity of plants were calculated in field trials and climate data including the averages of maximum temperature, minimum temperature, relative humidity and precipitation was compared with disease development in field trials. Increase in maximum temperature had strong positive correlation with disease severity, while relative humidity and precipitation were negatively correlated with disease development.

Keywords: *Solanum tuberosum*, Potato viruses, Viral Strain, Screening, Resistance.

INTRODUCTION

Potato is grown all over the world and potato viruses are very common in all potato growing countries of the world (Scholthof *et al*., 2011). Potato virus Y (PVy) is considered as the most important constraint among potato growers, it has occupied highest concern for potato industry worldwide (Kreuze *et al*., 2020).

PVY caused huge economic losses as in the seed crop, increase in every 1% incidence can result in loss of 180 kg/ hectare amounting to the revenue losses of up to $18 for each hectare (Nolte *et al*., 2004). Losses are not only limited to direct tuber loss but it has many other financial, social and environmental repercussions: increase in cost of production, management and control of virus affected fields, loss of valuable resources. In seed production the losses are even greater as it invites objections during inspection, certification and virus testing procedures (Lacomme and Jacquot, 2017; Ayana and Gabrekiristos, 2022). Although it is one of the old viruses of potato (de Bokx, 1981; Dykstra, 1939; Horváth, 1967; Hutton, 1945), but it has become relatively new devastating pathogen for Asian and sub continental potato crops (Ata-ul-Haq *et al*., 2016).

PVY have flexuous particles with diameter of 11-13nm and is about 700 nm long (Ayme *et al*., 2007). It is (+) sense single strand RNA with coat protein of the size of 30 kDa and its strand length has 9700 nucleotides (Barker *et al*., 2009). PVy genome forms poly proteins by 3000 to 3060 amino acids which converts to ten major mature proteins by viral encoded proteases.

Submitted: March 28, 2022
Revised: May 17, 2022
Accepted for Publication: June 5, 2022
* Corresponding Author:
Email: aropp2017@gmail.com

These proteins include CP, P1, P3, Nib, Cl, 6K1, 6K2 and NIa-Pro (Ayme *et al*., 2007: Revers and Gracia, 2015).

It's important for PVY virus to complete its life cycle by interacting with host proteins and subcellular organelles such as the ER or chloroplasts, as well as with other viral proteins, in order to carry out various essential functions (Ivanov *et al*., 2014). Cellular changes in the host plant can be seen using electron microscopy, with virions linked to Golgi apparatuses, plasmodesmata and the endoplasmic reticulum (Kerlan *et al*., 1999). Host mechanisms such as transmission and local movement for replication must be hijacked by PVy to express pathogenicity (Gebhardt *et al*., 2006). Potato Virus Y (PVY) exists in a complex of strains which can be noted in the field visually in the form of various foliar and tuber symptoms. These strains cause yield losses both in quality and quantity of tubers (Karasev and Gray, 2013). PVY strains were categorized on basis of symptoms which they produce in plants. Venial necrotic (VN) was named because it produced necrotic symptoms in tobacco plants (de Bokx and Hattinga, 1981). PVY^{C} and PVY^{O} produces mosaic symptoms in tobacco plants (Singh *et al*., 2008).

PVY^{Z} and PVY^{E} are not as common as PVY^{O} and PVY^{VN} but they can overcome resistance genes developed in plants against PVY^{O} and PVY^{VN} (Galvino-Costa *et al*., 2012). PVY^{O} strain assumed as the oldest one and later strains are proved as the recombinant isolates of these strains (Jakab *et al*., 1997; Boonham *et al*., 2002). Potato tuber necrotic strain (PVY^{NTN}) is relatively new strain and is characterized mostly by its ability to penetrate in the seed tubers of potato crop, it has better ability to remain latent and masked in tubers. It is assumed to be a recombinant of PVY^{Z} as the symptoms produced by (PVY^{NTN}) are more closely related to PVY^{Z} (Quintero-Ferrer *et al*., 2014).

Potato plants have developed resistance genes in response to these isolates, and N genes are the ones that provide resistance to PVY^{O} and PVY^{VN} and are common in Europe. Recombinant strains produced by PVY, on the other hand, are able to thrive in the wild, overcoming resistance genes. Three genes, Ryadg, Rysto, and Rychc, provide PVY with a hypersensitive resistance effect. Potato plants have a variety of genes that have evolved over time. None of above resistance genes have been conferred in varieties produced in Pakistan so far thus the management of PVY has been an extreme challenge for potato growers and seed producers of the country (Mughal *et al*., 2001). Vector management has proved as a tough challenge as it can be transmitted in a semi persistent manner by 65 species of aphids (Lacomme *et al*., 2017). And to add in constraints of management the climatic condition of major potato growing region is very conducive for aphid vector as temperature of these regions remains remain supportive for vector population. Evaluation of climatic conditions supportive for disease severity development is very important to intercept vector at crucial stages. Screening of varieties showing field resistance to PVY is very important for potato production and seed multiplication (Hühnlein *et al*., 2013). Thus the trial for screening of advance lines and potato varieties and evaluation of favorable environmental factors was conducted from 2018-19 to 2020-21 in experimental area Plant Virology Section, Plant Pathology Research Institute, Faisalabad.

MATERIALS AND METHODS

Plant Material: Potato advance lines and varieties, provided by Potato Research Institute Sahiwal on request of Plant Virology section were grown in field in 2018-21.

Micro propagation Technique: Healthy plants were conferred by Enzyme linked Immuno sorbant assay (Agdia, 2007). Apical part of healthy plants was taken to tissue culture lab of Plant Virology Section and after disinfection, were grown in Murashige and Skoog (MS) media. Plantlets were micro propagated for experimental purpose in test tubes for 3-4 week cycle (Hussey *et al*., 1981).

Growth Conditions: Plantlets were picked out of test tubes and were transferred in sterilized sand trays added with nitrogen fertilizer in controlled environment of 25°C. After 22 days plants were transferred in greenhouse in pots as shown in Figure 1.

PVY was preserved in tobacco plants in pots placed in insect free environment. Tobacco leaf tissues was used as inoculum, were homogenized in Phosphate buffer (50mM sodium phosphate, P^{H} 7.0). Diluted at rate of 1:10 (W:V) in pestle and mortar by using ice made by sterilized water.

Potato plants grown in insect free, temperature controlled greenhouse were rubbed with carborandum powder at 6-8 leaf stage and were inoculated by PVY virus suspension. Temperature of green house was maintained at 25°C.

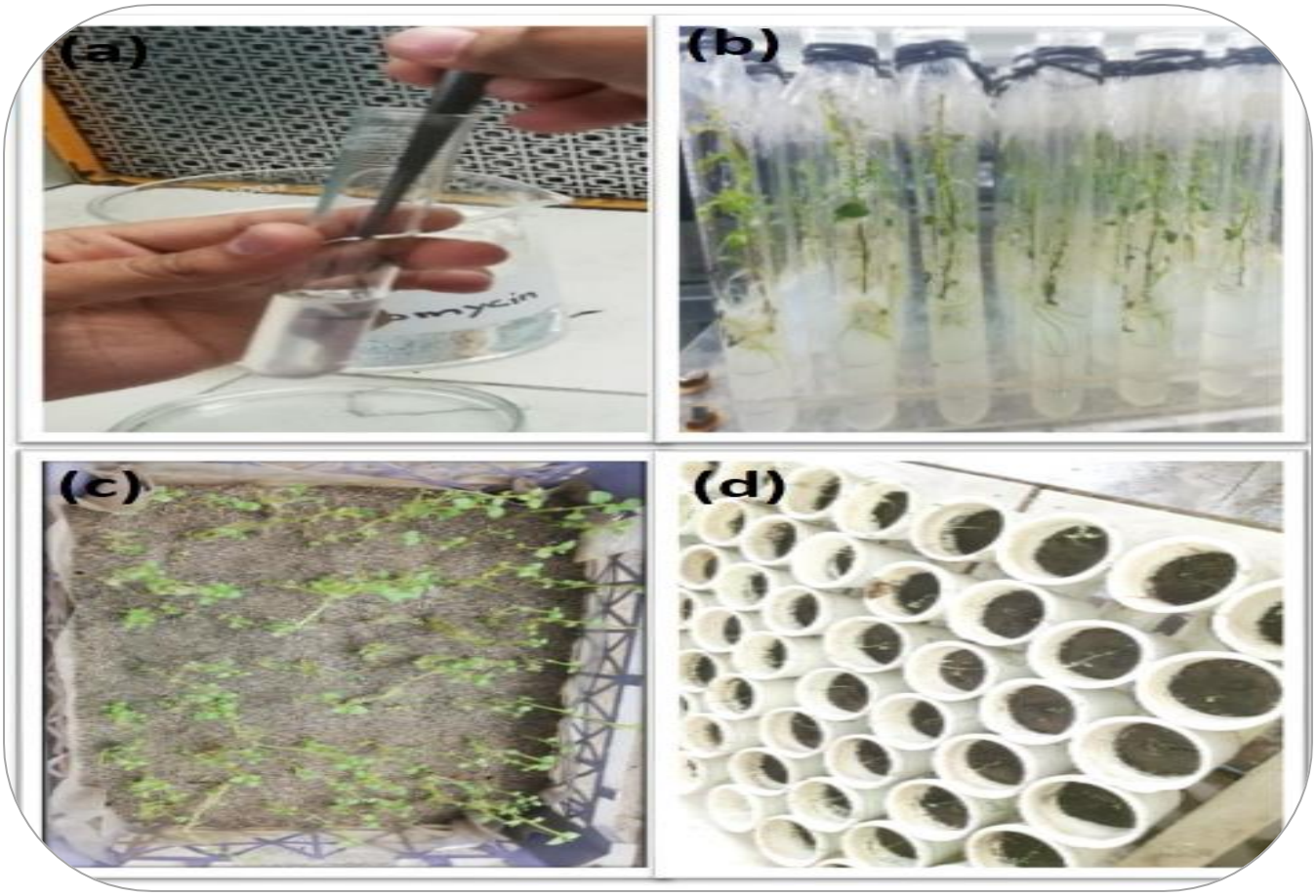

Figure 1. Tissue culture technique for production of virus free plants of Potato. (a). placement of apical portion of tissue on MS media, (b) growth of plantlets in test tubes, (c) transfer of plantlets in sand trays for acclimatization, (d) transfer of plantlets in experimental pots in green house.

Symptoms of plants were carefully observed on regular basis for lesion appearance, mosaic, crinkling and stunting, leaf dropping and erection. For confirmation of PVy symptoms five plants (3rd, 6th, 9th, 12th and 15th) from each line was subjected to ELISA test by using Direct Antibody Sandwich (DAS) ELISA (Clark and Adams 1977). Poly clonal antibody kit ("PVY poly" Art. No. 110572) made by BIOREBA was used for the purpose of detection of Antigen of PVY.

This Experiment was repeated thrice. *Cooke et al., (2006),* formula was used to record the incidence

$$\text{Disease Incidence of PVY} = \frac{\text{No. of infected plant units}}{\text{total number of plant units assessed}} \times 100$$

Table 1. For disease rating following scale of Mughal & Khan was used.

Disease rating scale	Reaction	Description
0	I	No Symptoms
1	R	Blackening and banding of veins on few leaves/Mosaic starting on all leaves
2	MR	Blackening and banding of veins on all leaves/Narrowing of leaves/ Venial necrosis severe mosaic/Leaf crinkling
3	MS	Rugosity and leaf drop streak, dwarfing
	S	Lower leaves dead, drooping, collapse of plants with very small tubers
5	HS	All leaves dead, stem dead or drying

I= Immune, HR= Highly Resistant, R= Resistant, MR= Moderately Resistant, MS= Moderately Susceptible, S= Susceptible, HS= Highly Susceptible.

For assessment of impact of environmental factors on varieties screened in field trials for three years, disease severity of plants in each variety was noted by visual observation bases by following formula of *Cooke et. al., (2006).*

$$\text{Disease severity of PVY} = \frac{\text{area of diseased tissue}}{\text{total tissue area}} \times 100$$

The reaction of verities was noted by using scale mentioned in Table 1. No line shown resistant or immune response neither line/ variety shown resistant response, 3 lines/varieties shown moderately resistant response, 5 varieties shown moderately susceptible response, 12 varieties shown susceptible response, 2 varieties shown highly susceptible response.

RESULTS

Table 2. Shows name of verities and respective response.

Disease rating scale	Reaction	Description	Name of V/L	No. of V/L
0	I	No Symptoms	-	-
1	R	Blackening and banding of veins on few leaves/Mosaic starting on all leaves		0
2	MR	Blackening and banding of veins on all leaves/Narrowing of leaves/ Venial necrosis severe mosaic/Leaf crinkling	PRI-RED, FD 7344 Cosmo	3
3	MS	Rugosity and leaf drop streak, dwarfing	FD 7349, FD 81-1, FD 74-38, FD 74-30, FD White	5
4	S	Lower leaves dead, drooping, collapse of plants with very small tubers	FD 74-28, FD 76-59, FD 76-55, FD 71-1, FD 1-3, FD 73-73, SL 28-72, SL 5-2, Ruby, SADAF, Sahiwal White, Sahiwal Red	12
5	HS	All leaves dead, stem dead or drying	SLI 04, SL 9-14	2

I= Immune, HR= Highly Resistant, R= Resistant, MR= Moderately Resistant, MS= Moderately Susceptible, S= Susceptible, HS= Highly Susceptible.

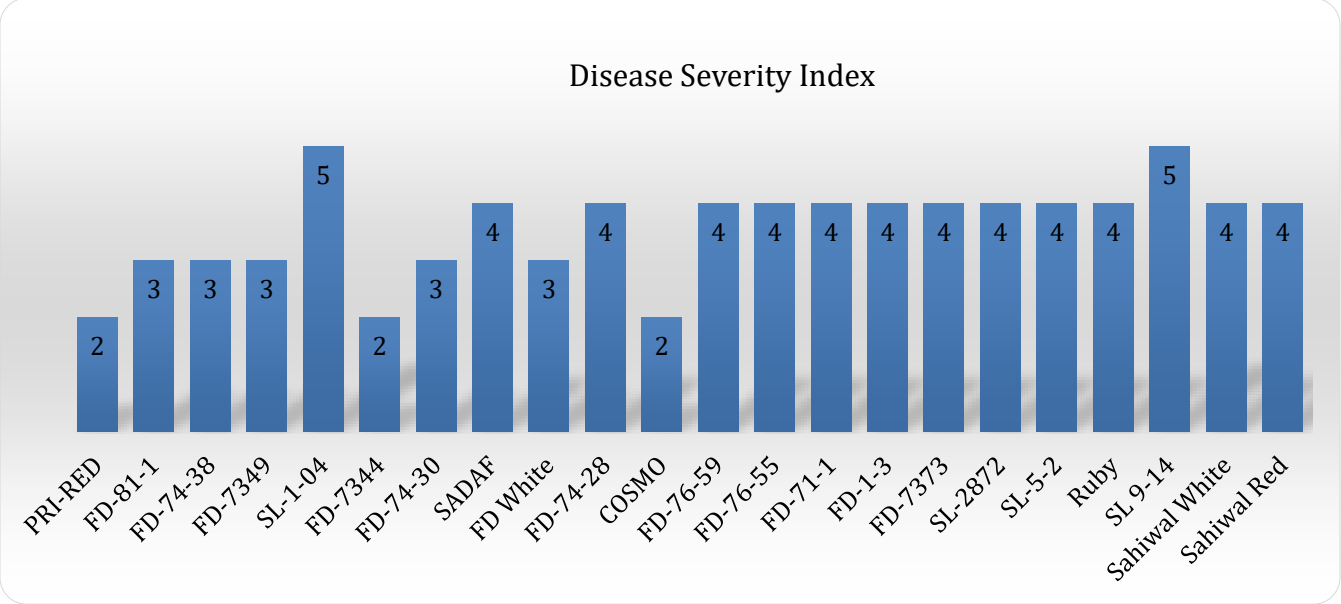

Figure 1. Disease scores based on severity of varieties grown in green house trial. Score 2: Moderately Resistant, Score 3: Moderately Susceptible, Score 4: Susceptible and Score 5 denotes for Highly Susceptible varieties.

ELISA RESULTS

ELISA was performed by providing overnight incubation period after each step. Plates of 96 wells produced yellowish color for strong antibody – antigen reaction denoting with concentration of virus titer present in samples. A strong reaction was noted as ++++, Plant material having medium concentration of titer produced light yellow color and was noted as ++ and + (Islam *et al.*, 2015).

Table 1. Antibody and Antigen reaction shown by yellowish color developed in ELISA plate.

Potato Line	Reaction	Potato Line	Reaction	Potato Line	Reaction
PRI-RED	+	FD White	++	SL-2872	+++
FD-81-1	++	FD-74-28	+++	SL-5-2	+++
FD-74-38	++	COSMO	+	Ruby	+++
FD-7349	+	FD-76-59	+++	SL 9-14	++++
SL-1-04	++++	FD-76-55	+++	Sahiwal White	+++
FD-7344	+	FD-71-1	+++	Sahiwal Red	+++
FD-74-30	++	FD-1-3	+++		
SADAF	++++	FD-7373	+++		

Some samples produced week reaction by producing unnoticeable color development but when the well plate was scanned on the ELISA scanner it was revealed there was weak reaction present in them as well. Light of 405nm was used to detect the antibody – antigen reaction.

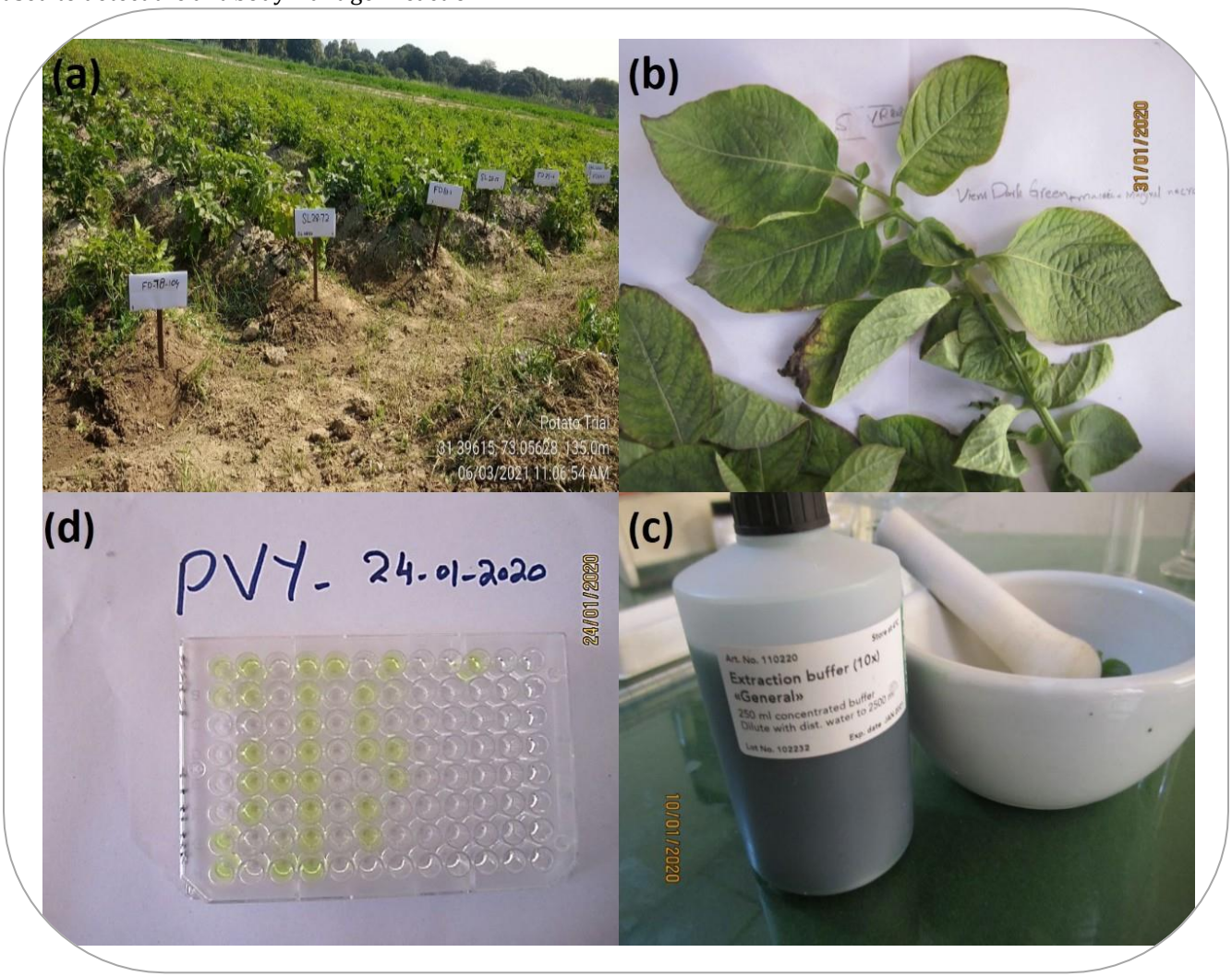

Figure 2. Procedure of ELISA. (a) Field trial for screening of potato crop. (b) Samples collection based on symptoms. (c) Loading of leaf samples in ELISA plate. (d) ELISA plate of PVy showing results with yellow wells as positive and transparent as negative.

Relation of Environmental factors with disease severity was calculated by analyzing the data of weekly averages of Maximum Temperature, Minimum Temperature, Relative Humidity and Precipitation.

Table 2. Statistical Relation of Environmental Data with Disease Severity Development of Potato lines.

		Avg T_MAX	Avg T_MIN	Avg RH%	Avg Precipitation
V1	Pearson Correlation	.911**	.817**	-.832**	-0.333
	Sig. (1-tailed)	0	0.001	0.001	0.158
V2	Pearson Correlation	.924**	.839**	-.854**	-0.341
	Sig. (1-tailed)	0	0.001	0	0.152
V3	Pearson Correlation	.984**	.925**	-.831**	-0.309
	Sig. (1-tailed)	0	0	0.001	0.177
V4	Pearson Correlation	.922**	.839**	-.860**	-0.351
	Sig. (1-tailed)	0	0.001	0	0.145
V5	Pearson Correlation	.973**	.914**	-.844**	-0.3
	Sig. (1-tailed)	0	0	0.001	0.185
V6	Pearson Correlation	.831**	.743**	-.793**	-0.179
	Sig. (1-tailed)	0.001	0.004	0.002	0.299
V7	Pearson Correlation	.981**	.923**	-.816**	-0.315
	Sig. (1-tailed)	0	0	0.001	0.173
V8	Pearson Correlation	.979**	.938**	-.826**	-0.271
	Sig. (1-tailed)	0	0	0.001	0.21
V9	Pearson Correlation	.980**	.922**	-.810**	-0.31
	Sig. (1-tailed)	0	0	0.001	0.177
V10	Pearson Correlation	.983**	.917**	-.856**	-0.363
	Sig. (1-tailed)	0	0	0	0.136
V11	Pearson Correlation	.985**	.906**	-.860**	-0.367
	Sig. (1-tailed)	0	0	0	0.134
V12	Pearson Correlation	.967**	.896**	-.842**	-0.35
	Sig. (1-tailed)	0	0	0.001	0.146
V13	Pearson Correlation	.967**	.943**	-.795**	-0.281
	Sig. (1-tailed)	0	0	0.002	0.201
V14	Pearson Correlation	.967**	.892**	-.873**	-0.353
	Sig. (1-tailed)	0	0	0	0.143
V15	Pearson Correlation	.979**	.911**	-.850**	-0.328
	Sig. (1-tailed)	0	0	0	0.162
V16	Pearson Correlation	.981**	.905**	-.836**	-0.376
	Sig. (1-tailed)	0	0	0.001	0.127
V17	Pearson Correlation	.990**	.940**	-.830**	-0.317
	Sig. (1-tailed)	0	0	0.001	0.171
V18	Pearson Correlation	.990**	.957**	-.812**	-0.302
	Sig. (1-tailed)	0	0	0.001	0.183
V19	Pearson Correlation	.981**	.934**	-.824**	-0.297
	Sig. (1-tailed)	0	0	0.001	0.187
V20	Pearson Correlation	.971**	.960**	-.791**	-0.29
	Sig. (1-tailed)	0	0	0.002	0.193
V21	Pearson Correlation	.973**	.929**	-.828**	-0.316
	Sig. (1-tailed)	0	0	0.001	0.172
V22	Pearson Correlation	.981**	.905**	-.836**	-0.376
	Sig. (1-tailed)	0	0	0.001	0.127

** represents highly significant relationship. Correlation of disease severity of Potato varieties to environmental factors. Avg T_Max: Weekly Average of Maximum Temperature. Avg T_Min: Weekly Average of Minimum Temperature. Avg RH%: Weekly Average of Relative Humidity. Avg Precipitation: Weekly Average of Precipitation during December to March 2021. V1=PRI-RED, V2=FD81-1, V3=FD 74-38, V4 FD 7349, V5=SL1-04, V6=FD 7344, V7=FD 7430, V8= SADAF, V9= FD White, V10= FD 7428, V11= COSMO, V12= FD 7659, V13= FD 7655, V14= FD71-1, V15=FD-1-3, V16= =FD 7373, V17= =SL 2872, V18= SL 5-2, V19= Ruby, V20=SL9-4, V21= Sahiwal White, V22= Sahiwal Red.

Correlation was calculated by using software SPSS, most of varieties shown highly significant positive correlation with increase in temperature and negative correlation was noted for relative humidity and precipitation (Table 4). Increase in temperature invited more aphid population during the month of February.

Disease Severity was calculated during three crop seasons for evaluating the crop stand losses and it was observed that maximum disease severity was noted on SL 1-04 in 2019 , SL 9-14 in crop season of 2020 and again in SL 1-04 in 2021.

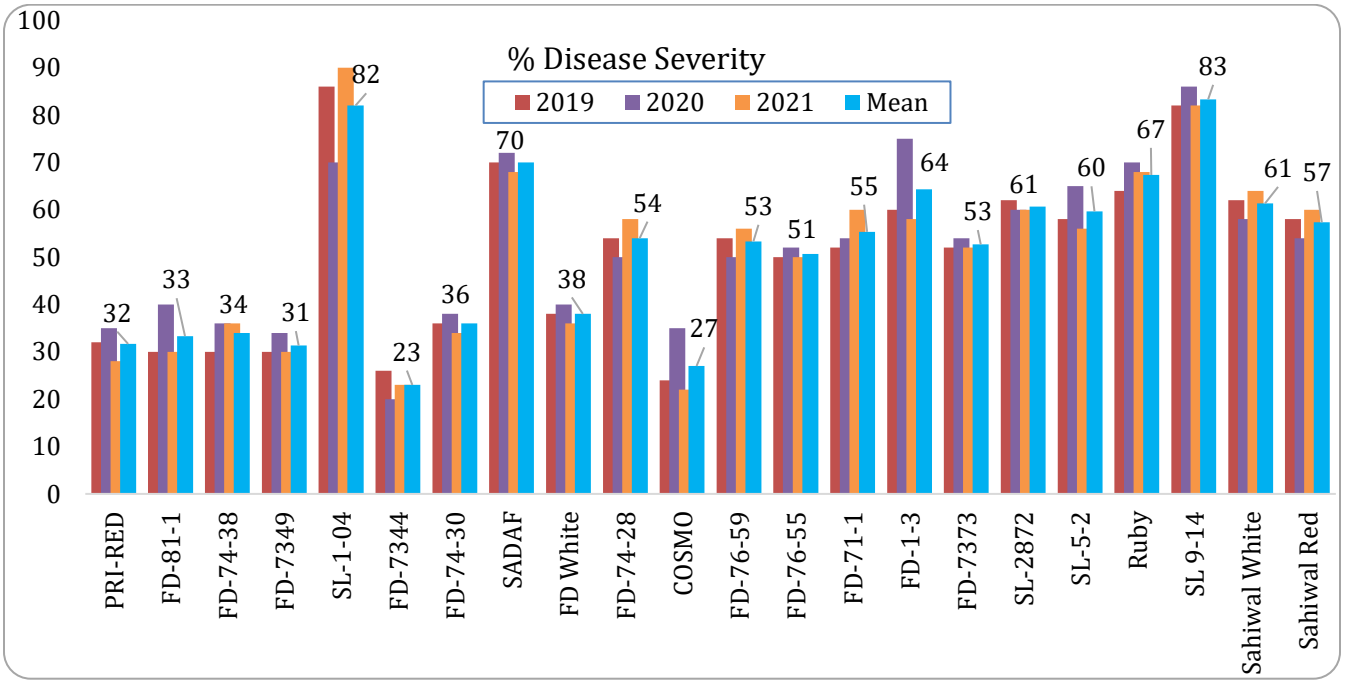

Figure 3. Disease Severity during three cropping seasons

Potato Virus y strains was tested and two strains PVY^{O} and PVY^{VN} were found most frequently. PVY^{O} and PVY^{LD} are found in Pakistan from last four decades but from few years very high incidence of PVY^{VN} strain is an emerging issue.

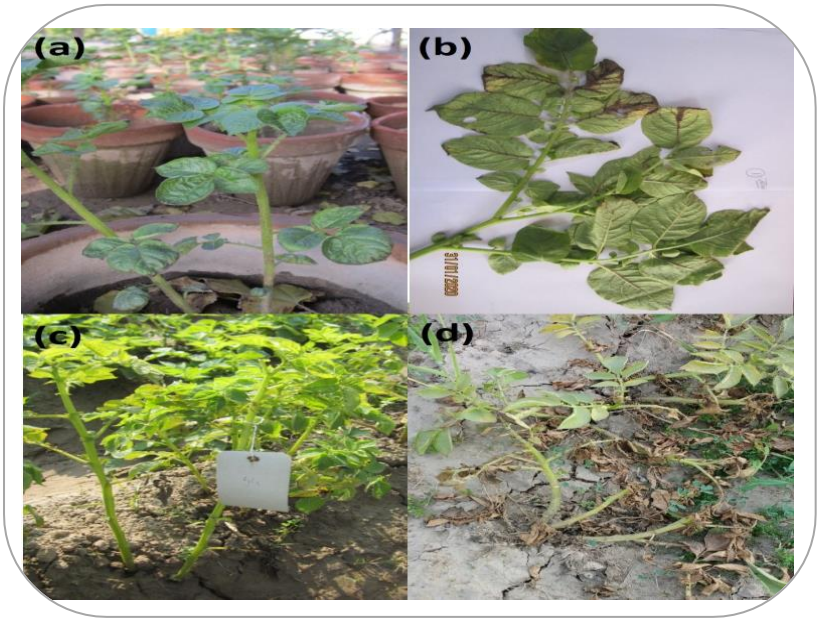

Figure 4. PVY^{O} PVY^{LD} and PVY^{VN} strains on Potato plants. (a) symptoms of PVY^{VN} strain on leaves of potato. (b) veinal necrosis of PVY^{VN} strain on lower side of leaves. (c) PVY^{LD} strain of PVy, lower brach of stems drop and gets brown. (d) Late stage of PVY^{O} , stems dying.

For identification on PVY^{VN} strain a careful observation of viens on lower side of leaves and mosaic is highly recommended. At later stage vienal necrosis turns brown corky however use of monoclonal ELISA kit was found accurate for PVY^{O} and use of poly clonal anibody ELISA kit was found very helpful for detection of most PVy strains.

Potato Virus Y is prevalent in all the potato varieties/ lines currently grown on Pakistani fields. No variety has shown any promising resistance in field thus there is a dire need to carry along the management strategies such as integrated disease management programs. Molecular identification of N and R resistant gene for selection of line for crossing during variety development can be used by biotechnological methods. Seed of potato used for ration production should be obtained from a verified source and farmers are advised to avoid cutting the tubers for planting purpose as it has more probability to transmit virus in healthy tubers. Control of vector is very important in higher temperatures as the results revealed that severity increases with increase in temperature and thus defoliation and drying of stems results in significant yield loss.

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Contribution of Authors:

Muhammad Ussama-Yasin	:	Conducted Trials, Performed ELISA, Tissue culture and Written Manuscript
Muhammad Burhan	:	Provided Technical Assistance
Azhar Mustafa	:	Supervised Trials, Lab Studies and Provided All resources for study
Shaukat Ali	:	Provided Technical Assistance
Salman Ghuffar	:	Provided Technical Assistance
Waseem Abbas	:	Provided Technical Assistance
Muhammad Z. Niaz	:	Provided Technical Assistance
Tariq Javaid	:	Provided seed and Advisory Assistance