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Research Article

SCREENING OF POTATO GERMPLASM FOR RESISTANCE TO POTATO VIRUS Y AND POTATO LEAFROLL VIRUS USING DAS-ELISA

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ABSTRACT

Potato (*Solanum tuberosum* L.) ranks as the fourth most important staple food globally, after rice, wheat, and maize. In Pakistan, *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV) are among the most critical pathogens affecting potato crops, causing yield losses of up to 90%. Fifteen advanced potato lines from the Potato Program of the Horticultural Research Institute (HRI) were screened for resistance to these economically significant viruses. The experiment was conducted in the glasshouse of the Crop Diseases Research Institute, NARC, Islamabad, during 2023-24, following a completely randomized design with three replications per clone. For screening, small tubers of fifteen potato clones, including one check variety, were planted in pots within the glasshouse. PVY was mechanically transmitted, while a chip graft inoculation assay was used for screening against PLRV. Symptoms began to appear two weeks after inoculation, and data were recorded four weeks post-inoculation. A double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed on all plants to confirm the presence of both viruses serologically. The findings revealed that the line HRI-P1 remained asymptomatic and was categorized as highly resistant to both viruses, based on symptom observation and ELISA testing. Six clones exhibited varying levels of resistance, three showed moderate susceptibility, one was susceptible, and three were highly susceptible to PVY. The study highlights the availability of valuable sources of resistance within the Potato Program. HRI, NARC, which could be utilized in breeding potential future varieties. The disease-resistant genotypes identified will be instrumental in future potato breeding programs.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most widely produced and consumed tuber crops globally. Likely originating in Peru (South America), the potato is believed to have spread to other parts of the world through military expeditions, trade, and transportation (de Haan and Rodriguez, 2016). Today, over 5,000 potato varieties are cultivated worldwide. Potatoes are a vital staple food

crop for humans but face several yield-limiting factors, with disease susceptibility being a primary concern (Burgos et al., 2020). In Pakistan, potatoes are a key cash crop for farmers and contribute significantly to the Gross Domestic Product of the country. They are grown both as garden vegetables for personal consumption and on a commercial scale, highlighting their importance in economic activity and food production. During the 2022-23 period, potatoes were cultivated on 341,000 hectares, with an annual production of 8.319 million tons in Pakistan (MNFSR, 2022-23).

A major constraint to yield reduction is the impact of various biotic and abiotic factors on potato crops during the growing season (Nisa et al., 2022). Over 50 different viruses and one viroid have been reported to infect potatoes worldwide, reducing yield and quality. Currently, Potato Virus Y (PVY) and Potato leafroll virus (PLRV) are the most detrimental viruses affecting potatoes globally, with PVY now considered the foremost threat (Kreuze et al., 2020). PVY has been particularly challenging over the past 20 years due to the emergence of recombinant PVY variants. Infection with PVY or PLRV can lead to the downgrading or rejection of seed lots if the infection level exceeds the tolerance thresholds set by seed potato certification schemes (Krijger et al., 2020). It is estimated that using seeds infected by these viruses can lead to a threefold decrease in average potato yield. Infected plants generally produce smaller tubers and lower yields. When these tubers are used for reproduction, they lead to the growth of diseased plants and a decline in crop quality (Ciric et al., 2018). In Pakistan, yield losses are often attributed to various viruses (Hameed et al., 2014). In spring, summer, and autumn potato crops, viruses such as PVY, PLRV, Potato Virus A (PVA), Potato Virus M (PVM), Potato Virus S (PVS), Potato Virus X (PVX), and Potato Mop-top Virus (PMTV) have been reported. These viruses can contribute to yield losses of up to 83% in affected crops.

PVY, belonging to the family Potyviridae, includes the most economically significant and largest group of plant viruses. PVY is a single-stranded RNA virus with several strains, including PVY^{N} (tobacco vein necrosis strain), PVY^{O} (common strain), and PVY^{C} (stipple streak strain). Recently, recombinant strains derived primarily from PVY^{N}, such as $PVY^{N\text{-}Wi}$, PVY^{NTN}, and PVY^{N} have emerged in potato crops (Buchen-Osmond, 1987). PVY is transmitted both mechanically and through insect vectors in a nonpersistent manner; the virus can be acquired by the vector in minutes and transmitted within seconds (Burrows and Zitter, 2005). PLRV is transmitted by various aphid species, with *Myzus persicae* (family: Aphididae) being the most efficient vector, transmitting the virus in a persistent, non-propagative manner. In Pakistan, the prevalence of *M. persicae* was first reported in 1978 (Abbas et al., 2020). PLRV, transmitted by *M. persicae*, can cause yield losses of 40-70%. To address this issue, germplasm from the Potato Program at the Horticultural Research Institute, NARC, was screened for disease-resistance or tolerance and susceptible genotypes. The identification of disease-resistant genotypes through ELISA will aid in the future introgression of resistant genes into susceptible potato cultivars as part of a breeding program.

MATERIALS AND METHODS

Plant material

Plant materials for this study was obtained from the Potato Program, Horticultural Research Institute, National Agricultural Research Center (NARC), Islamabad (Latitude: 33.6982°N, Longitude: 73.0393°E) during the 2023-24 growing season. Fifteen different local and exotic potato clones, including HRI-P1, HRI-P2, Exotic Potato IV, Exotic Potato V, CIP-19, SH-5 × Axona 7, FD-110 × SH-5, NARC-2008-H, Horizon × Sarpomira, NARC-2002-1 × Axona-17, CIP-25, 9619 × SH-5, Blue Denubi × Axona-10, NARC 17-19 × Axona-10, and Blue Denubi × Axona-15, were selected for screening against two economically significant viruses: PVY and PLRV. A commercially grown, highly susceptible variety, Leady Rosetta (LR), was used as a control. The exotic germplasm was imported from the International Potato Center (CIP) in Lima, Peru. Preliminary visual screening of the selected clones was conducted in the field based on symptomatology.

To confirm the presence or absence of the viruses, small tubers from each clone, including the LR control, were planted in pots within the glasshouse of the Crop Diseases Research Institute, NARC, Islamabad, in autumn 2023. The experiment was designed as a Completely Randomized Design (CRD) with three replications per clone. The temperature was maintained between 22-28°C during the day and 14-20°C at night, with ambient lighting and humidity levels throughout the study. Six plants (five inoculated and one control) from each clone were selected based on their growth for further screening against PLRV and PVY.

Phenotyping assay for PVY

Mechanical inoculation method was employed to screen potato germplasm for resistance to PVY. Five plants from each clone were individually inoculated with PVY. The PVY inoculum was collected 45 days after sowing from pre-infected leaves grown in the screen house of the Crop Diseases Research Institute, NARC, and were confirmed PCR positive for PVY as shown in Figure 1a. For inoculum preparation, infected leaves were collected, and a 1 g sample was crushed in 10 ml of phosphate buffer. The extraction buffer was prepared by dissolving 7.5 g of $NaH_2PO_4 \bullet 2H_2O$ (0.05 M phosphate buffer) and 1% (w/v) Na_2SO_3 in 1 L of distilled water, with the pH adjusted to 7.4. An inoculum concentration of 1 µg was prepared, and 500 µl of this solution was applied to each plant for mechanical inoculation. While five plants were inoculated with the PVY inoculum, one plant was left uninoculated as shown in Figure 2b.

Two leaves per plant were selected for artificial inoculation. The selected leaves were powdered with carborundum powder to facilitate virus transmission, and the PVY inoculum was then gently rubbed onto the leaves. Two days after the initial inoculation, a second dose of PVY inoculum was applied to the plants.

Plants were tested for virus infection using enzyme-linked immunosorbent assay (ELISA) by taking leaf samples four weeks post inoculation. Clones in which the virus was detected were declared as susceptible, while those, in which no virus was detected, were stated as resistant.

Phenotyping assay for PLRV

For screening against PLRV, the chip grafting inoculation assay was employed. This technique involves grafting a small piece of tissue (a chip) from a virus-infected plant onto a healthy plant. Plants already infected with PLRV which were confirmed PCR positive (for PLRV), as shown in Figure 1b, were used as a source for chip graft. Fifteen potato clones were selected for screening against PLRV. A small chip, approximately 2 cm in size, was cut from the infected plant, while a corresponding vertical cut was made on the healthy clones, into which the chip was inserted to ensure good contact between the tissues. The grafted area was then wrapped with parafilm to secure the chip in place. Parafilm was expired after ten days post inoculation. Symptoms were observed on regular intervals post graft inoculation. Data was collected four weeks post graft inoculation by observing PLRV symptoms such as leaf rolling and stunted growth, followed by an ELISA test to confirm the presence of the virus. Clones in which the virus was detected were deemed susceptible, while those in which the virus was absent were considered resistant.

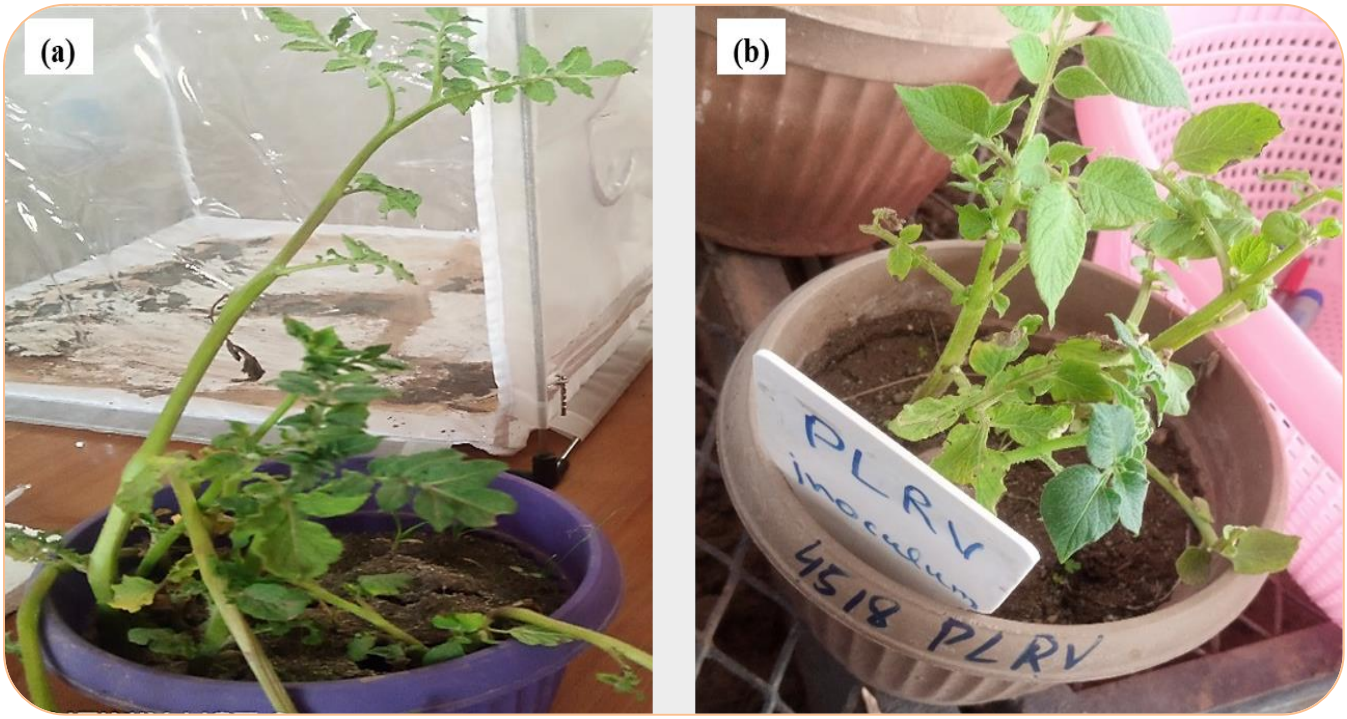

Figure 1. (a) Infected potato plant with PVY. (b) Infected potato plant with PLRV.

Figure 2. (a) Potato clones before inoculation. (b) Potato clones after inoculation.

Double antibody sandwich enzyme linked immuno sorbant assay (DAS-ELISA) test for viruses

Infected potato leaves were tested for the presence of viruses using the DAS-ELISA technique. Commercial DAS-ELISA Kit for PVY from BIOREBA was used and standard protocol was followed, and major steps are stated here. The PVY specific antibody was diluted 1:1000 in coating buffer and coated to the ELISA plate. The plate was incubated at 4°C for 24 h, followed by three washes with wash buffer of PBST. Samples were extracted in phosphate buffer and the antigen sap was placed in each well of the ELISA plate as per the layout plan. The plate was incubated overnight at 4°C. After incubation, the wells were washed three times with wash buffer. A 1:1000 dilution of the antiviral solution containing the enzyme conjugate was added to each well, and the plate was covered and incubated at 37°C for 2-4 hours. Para-nitrophenyl- phosphate (PNP) tablets were used to prepare the fresh substrate, and after dispensation to each well, plate was incubated for 30-60 min at 37°C. The results were assessed by spectrophotometric measurement on the Multiskan™ GO Microplate Spectrophotometer at 405 nm absorbance.

Statistical analysis

The experimental data were analyzed using R Studio, and a graph illustrating disease severity was prepared using Excel.

RESULTS

Screening of potato varieties/clones against PVY

Symptoms started appearing two weeks post inoculation in case of PVY. Mild to severe mosaic with yellowing was observed. Symptomatic and serological testing revealed that some potato clones were infected with PVY, as shown in Figure 3. The results indicated that among all the clones, HRI-P1 exhibited high resistance to PVY, with a disease severity index (DSI) of 0.2, as shown in Table 1. HRI-P2 and CIP-19 also displayed resistance to PVY, with DSIs of 1.4 and 1.6, respectively. Clones such as Exotic Potato IV, NARC-2008-H, NARC-2002-1 × Axona-17, 9619 × SH-5, Blue Danube × Axona-10, and Blue Danube × Axona-15 showed moderate resistance to PVY, with DSIs of 2.4, 2.2, 2.2, and 2.0, respectively. The clone Horizon × Sarpomira was found to be moderately susceptible, with a DSI of 2.6, followed by CIP-25 and NARC 17-19 × Axona-10, both with DSIs of 2.6. Clones such as Exotic Potato V, SH-5 × Axona 7, and FD-110 × SH-5 were identified as highly susceptible. ELISA results confirmed the presence of the virus in susceptible clones, while no virus was detected in resistant ones, as indicated by the '+' and '-' signs in Table 1.

Screening of potato varieties/clones against PLRV

In case of PLRV, leaf rolling started appearing three weeks post inoculation which is late compared to PVY. The results

showed that out of fifteen clones/approved varieties, three clones, HRI-P1, CIP-25, and Blue Danube × Axona-10, demonstrated high resistance to PLRV, with DSIs of 0.2 and 0.4, as shown in Table 1. HRI-P2 and FD-110 × SH-5 also exhibited resistance, each with a DSI of 1.4. Clones such as Exotic Potato IV, CIP-19, NARC-2008-H, NARC-2002-1 × Axona-17, and NARC 17-19 × Axona-10 displayed moderate resistance, with DSIs of 2.4 and 2.0. Exotic Potato V, SH-5 × Axona 7, Horizon × Sarpomira, and Blue Danube × Axona-15 were found to be moderately susceptible. Only one germplasm, 9619 × SH-5, was found to be susceptible to PLRV, with a DSI of 4. ELISA results confirmed the presence of PLRV in susceptible clones, while no virus was detected in resistant ones, as indicated by the '+' and '-' signs.

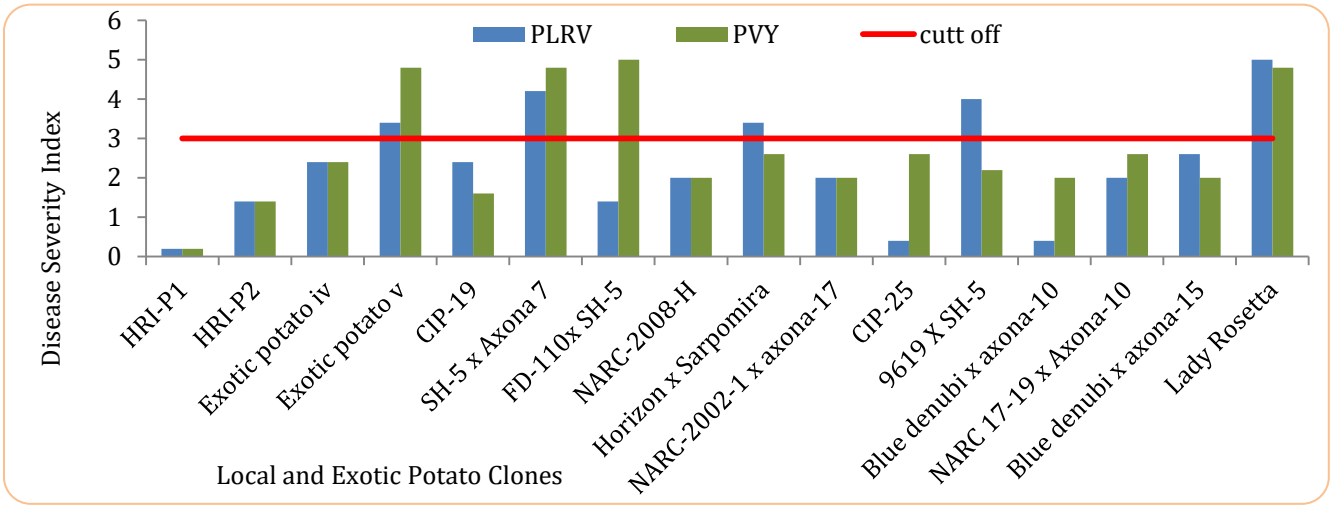

Figure 3. Response of different potato clones against PVY and PLRV.

Table 1. Results of enzyme linked immunosorbent assay (ELISA) of two viruses.

S. No.	Germplasm ID	PLRV			PVY		
		DS1	ELISA Results	Reaction	DS1	ELISA Results	Reaction
1	HRI-P1	0.2	-	HR	0.2	-	HR
2	HRI-P2	1.4	-	R	1.4	-	R
3	Exotic potato iv	2.4	-	MR	2.4	-	MR
4	Exotic potato v	3.4	+	MS	4.8	+++	HS
5	CIP-19	2.4	-	MR	1.6	-	R
6	SH-5 × Axona 7	4.2	+	MS	4.8	+++	HS
7	FD-110 × SH-5	1.4	-	R	5	+++	HS
8	NARC-2008-H	2	-	MR	2	-	MR
9	Horizon × Sarpomira	3.4	+	MS	2.6	+	MS
10	NARC-2002-1 × axona-17	2	-	MR	2	-	MR
11	CIP-25	0.4	-	HR	2.6	+	MS
12	9619 × SH-5	4	++	S	2.2	-	MR
13	Blue denubi × axona-10	0.4	-	HR	2	-	MR
14	NARC 17-19 × Axona-10	2	-	MR	2.6	+	MS
15	Blue Denubi × axona-15	2.6	+	MS	2	-	MR
16	Lady Rosetta	5	+++	HS	4.8	+++	HS

DISCUSSION

Current study was conducted to screen the potato germplasm available at the Potato Program, Horticultural Research Institute, National Agricultural Research Center, Islamabad, Pakistan, for resistance to two of the most devastating viruses; PVY and PLRV. The screening process involved artificial inoculation of the plants, followed by noting phenotypic disease expression and quantitative ELISA to detect the presence of the viruses. Mechanical inoculation method and chip grafting inoculation assay were employed for inoculation of PVY and PLRV, respectively.

The results indicated that the germplasm lines HRI-P1 and HRI-P2 exhibited resistance to both viruses. In contrast, Exotic Potato IV, NARC-2008-H, and NARC-2002-1 × Axona-17 demonstrated moderate resistance. Horizon × Sarpomira was found to be moderately susceptible to both PVY and PLRV. Exotic Potato V and SH-5 × Axona 7 were moderately susceptible to PLRV and highly susceptible to PVY. CIP-25 showed high resistance to PLRV but was moderately susceptible to PVY. The line 9619 × SH-5 exhibited susceptibility to PLRV while showing moderate resistance to PVY. The check variety, Lady Rosetta, was found to be highly susceptible to both viruses.

These findings are consistent with those of Arifa et al. (2020), who also screened various potato germplasm using the ELISA technique. Similarly, Jarjees (2000) utilized ELISA for the rapid identification of PVY, reporting significant reliability of ELISA with reference to potato screening. Potato viruses are a major cause of yield losses in Pakistan, underscoring the importance of ongoing screening efforts. Ashraf et al. (2020) also conducted a serological assay (DAS-ELISA) to screen potato germplasm against PVY and it was helpful to find the resistance source. In Ethiopia, Tessema et al. (2024) used the DAS-ELISA method to detect different viruses in potato varieties, confirming widespread latent virus infections in early-generation potato seeds, which restrict potato production.

Further supporting evidence comes from Islam et al. (2015), who screened potato germplasm for various viruses using ELISA and it was found to be an effective technique for screening against viruses. Yardimci et al. (2018) detected PVY, PVX, PVS, PVA, and PLRV in different potato varieties by testing 278 samples with the DAS-ELISA method, finding that both tubers and leaves were infected due to vegetative propagation. In Egypt, Araby et al. (2009) conducted serological testing, revealing that out of 26 potato samples, 10 were infected with PLRV, 7 with PVY, and 4 with PVX. Karpova et al. (2019) also analyzed potato leaf samples using ELISA, detecting viruses such as PVM, PVS, PVY, PVX, and PLRV, with 84.3% of the samples infected with PVM and 46.6% with PVS.

CONCLUSION

Potato Virus Y and Potato Leafroll Virus are among the most devastating viruses affecting potato crops in Pakistan. To address this challenge, a study was conducted to screen potato germplasm available at the Potato Program, HRI, NARC, Islamabad. Various local and exotic potato clones were screened using mechanical inoculation method, coupled with the serological technique DAS-ELISA for virus detection. Mechanical transmission was employed for PVY, while chip grafting inoculation assay was exploited for PLRV. The results revealed that among all the potato clones tested, HRI-P1 and HRI-P2 demonstrated resistance, while Horizon × Sarpomira was found to be susceptible to both viruses. Other clones exhibited varying levels of resistance and susceptibility. The identified resistant germplasm will be utilized in future breeding programs to develop disease-resistant varieties.

By identifying and cultivating potato varieties that are resilient to prevalent diseases, we can significantly boost yields and reduce crop losses. Disease-resistant clones can lead to more sustainable farming practices, reduce reliance on chemical pesticides, and improve economic outcomes for farmers. Moreover, the study highlighted that performing initial screenings in a controlled and consistent setting allows for a more focused and manageable evaluation of the clones' resistance traits. This approach establishes a baseline understanding of each clone's performance, enabling researchers to identify promising candidates for further, more comprehensive multi-location and multi-year trials. This study also indicates that a valuable source of resistance is available locally at the Potato Program, HRI, NARC, and these resistant clones are recommended for use in future breeding programs.

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germplasm. Thanks are also extended to the Crop Diseases Research Institute at the National Agricultural Research Center, Islamabad, for their collaboration and conduction of the experiment.

AUTHORS' CONTRIBUTIONS

QN, AR, MAF, and MH designed and formulated the study; QN, AR, AM, MU, and RMMN conducted the experiments; MZ and MAF provided necessary financial and material resources; MAF, AR, AM and QN collected, arranged and analyzed the data; WAD provided technical assistance; MZ and MH supervised the work; QN wrote the manuscript; AR, TB and SSA proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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