

Screening of Potato Germplasm through ELISA against Potato Virus X (PVX)

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Abstract Ten varieties / lines of potato germplasm were screened out against PVX to find the resistant source. Out of 10, four varieties were susceptible, three moderately susceptible and three were moderately resistant. DAS-ELISA detected the PVX in leaves samples of different Potato varieties. Mechanical transmission produced the local lesion and insect transmission through aphid (*Myzus Persicae*) was failed due to unknown reasons.

Keywords Potato Varieties, PVX, DAS- ELISA, Transmission

PVX is transmitted mechanically. Insect transmission is not known yet. It is immunogenic and easily detected by different serological tests as ELISA and time-resolved fluoroimmunoassay [13]. In Pakistan PVX has been reported and detected through ELISA [6]. Because of latent nature of PVX, ELISA is a reliable, quick and cheap method for its detection. Insect transmission of PVX is still a question mark. Hence this research was carried out to find out the resistant source from germplasm, its transmission and detection through ELISA.

1. Introduction

Potato (*Solanum tuberosum* L.) is a valuable perennial plant belongs to family *Solanaceae*. It is ranked 4th in production after wheat, rice and maize. Pakistan has been divided into eight agro ecological zones for potato production. The main hub for seed production is northern areas from where seed is provided rest of the country [5]. In Pakistan, average yield is low as compared to other countries in the world, due to under attack of various pathogens like bacteria, nematode, fungi and viruses [11]. Among them viral diseases caused by viruses such as; Potato virus X (PVX), Potato leaf roll virus (PLRV) and Potato virus Y (PVY) are most devastating pathogens prevail in potato field in Pakistan. Losses due to these viruses are upto 83% [10].

PVX is widely distributed not only in Pakistan but worldwide with wide host range. It belongs to *Potexvirus*, family *Alphaflexiviridae* virus. It is rod shaped, filamentous, monopartite +ve sense ssRNA virus. PVX alone can cause yield loss in the range of 5-20% depending upon the potato genotype, virus strain and the simultaneous infection with other viruses like PVY and PVA [2]. The major symptoms of PVX are inter venial mosaic, rugosity, stunting, mottle on upper leaves and small sized tubers. It is also called mild mosaic virus due to mild or no symptom in most potato varieties but has synergistic effect with PVY resulting in severe symptoms. Because of latent infection PVX is difficult to be characterized.

2. Materials and Methods

2.1. Screening of Potato varieties / lines against PVX

Ten varieties, (Tota 704, FD 71-1, FSD White, FD 74-41, FD 74-50, SH 216-A, N 96-25, Kuruda, FD 8-1 and FD 76-24) were screened out against PVX to record the disease incidence and severity index.

Disease incidence was calculated after the confirmation through ELISA. Samples from suspected plants to be infected were collected on the basis of symptoms (fig.1).

$$\text{Disease Incidence} = \frac{\text{No. of infected plants}}{\text{Total plants observed}} \times 100$$

Disease rating scale developed by Mughal and Khan [9] with slight modification was used to record the disease severity.

2.2. Disease Severity Index (DSI)

Grade	Rating Scale	Reaction
0	No visible symptom	HR
1	Mild mottling on the upper leaves	H
2	Intervinal mosaic symptoms on more than one leaf	MR
3	Mosaic symptoms on all leaves	MS
4	Distinct mosaic symptoms on all leaves	S
5	All above symptoms and small number of sized tubers	HS

2.3. Double Antibody Sandwich ELISA

Potato leaves samples were subjected to be DAS- ELISA [4] for the confirmation. Followings are different steps in DAS-ELISA;

1. ELISA plate was coated with coating buffer containing IgG diluted @ 1:1000.
2. The plate was incubated at 4°C for 24 hours followed by washing three times with PBS-Tween buffer.
3. Antigen (sap) extracted in phosphate buffer was charged in each well of ELISA plate.
4. Step 2 was repeated.
5. 200ul Anti-virus conjugate dilution @ 1/1000 in conjugate buffer was added to each well.
6. Step 2 was repeated.
7. Substrate buffer containing pNP was added to each well and incubated at room temperature for half an hour.
8. Result was assessed visually.

2.4. Mechanical Transmission

0.05 M Phosphate buffer at (pH 7.6) was used for grinding young virus-infected tissue to prepare inoculum. The indicator plants were raised in small pots. Carborundum powder (600 meshes) was dusted on leaves of indicator plants. Crude saps were applied on the leaves with the help of forefinger and plants were rinsed off with distilled water immediately after inoculation to remove the excess sap. These plants were kept in an insect free box to observe the symptoms for 2 -3 weeks.

2.5. Insect Transmission

Green peach aphids (*Myzus persicae* Sulz.) were removed for the healthy potato plants and starved in dark for two hours in a sealed container. The aphids were first transferred to infected potato leaves for half an hour and then transferred to indicator host plants. Plants were kept in insect free chambers.

3. Results

3.1. Screening of Potato Varieties / Lines Against PVX

Disease incidence and disease severity was recorded in the figure, 1 and 2. Disease incidence was ranged from 37 to 60% (Fig.2). On the basis of disease rating scale the varieties were grouped into three categories; susceptible with DSI 4 (FD 74-41, SH 216-A, N 96-25, FD 8-1), moderately susceptible with DSI 3 (FD 71-1, FSD White, FD 74-50) and moderately resistant with DSI 2 (Tota 704, Kuruda, FD 76-24), (fig, 3).

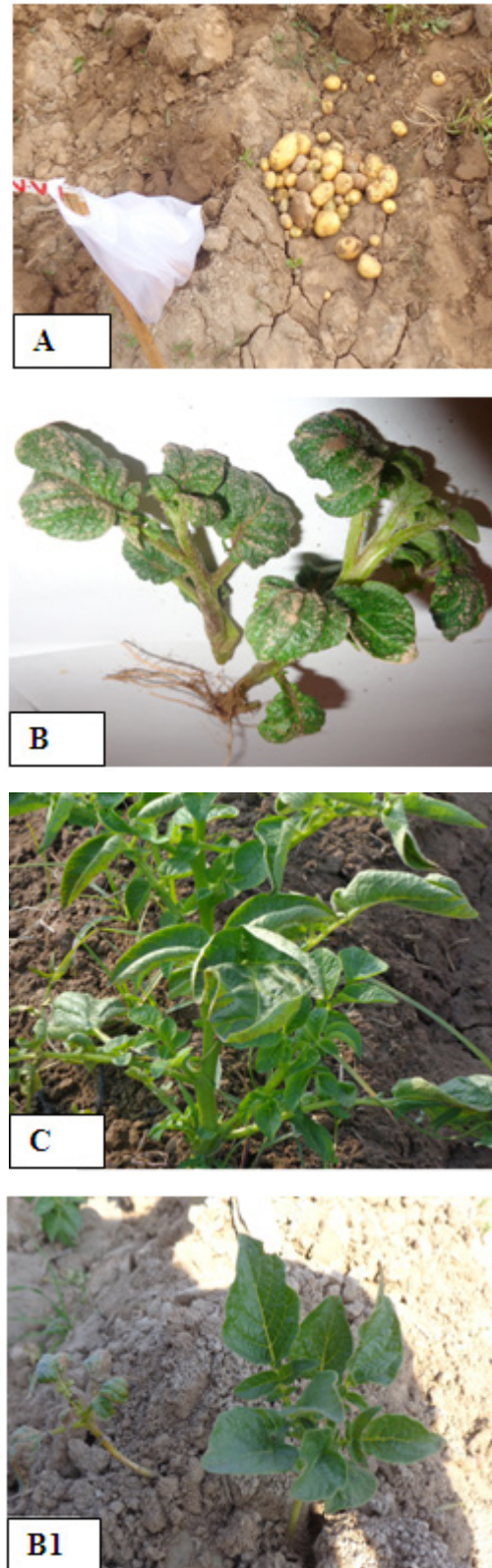
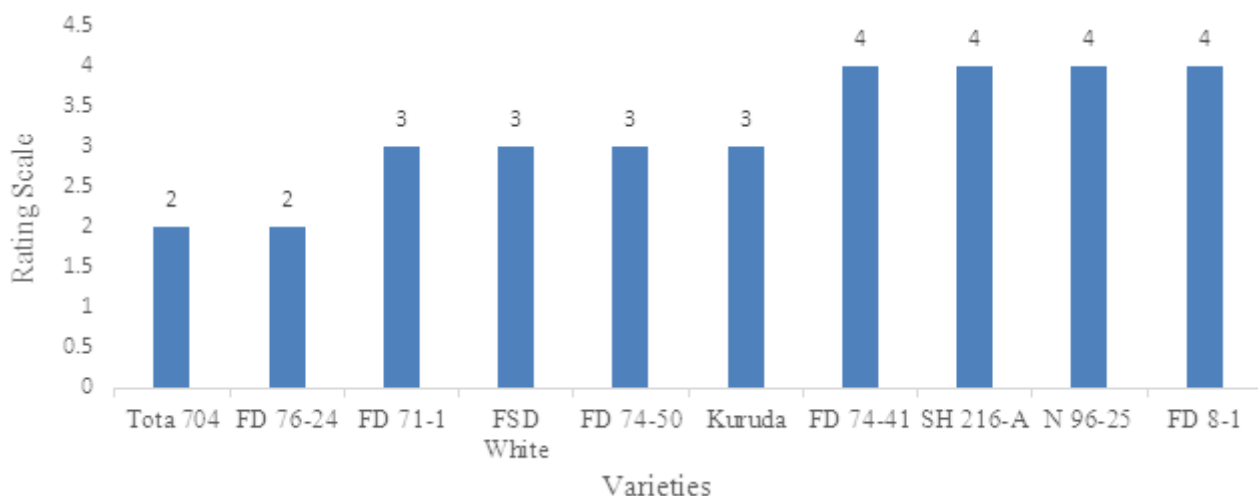


Figure 1. Symptoms of PVX (A) Small sized tuber. (B, B1) Stunted plant. (C) Rugosity



Figure 2. Incidence of PVX in different Potato varieties



2 = Moderately Resistance (MR), 3 = moderately susceptible (MS), 4 = Susceptible (S)

Figure 3. Response of potato varieties against PVX

3.2. Serological Indexing

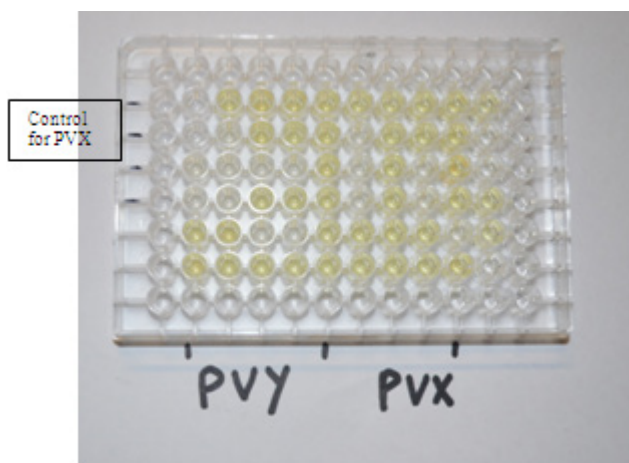


Figure 4. ELISA plate showing yellow and no colors which are indications of Presence or absence of virus respectively

DAS-ELISA confirmed the PVX in the collected samples from different potato varieties. Positive samples showed

moderate yellow colour (Fig.4).

3.3. Transmission:

Mechanical inoculation of PVX produced local lesions. Among four indicator plants *Spinacia oleracea* did not produce local lesions (Table 1). Mechanical inoculation was also confirmed through ELISA. The aphids were failed to transmit the PVX.

Table 1. Mechanical transmission on indicator plants

Indicator Plant	No. of Plant	Infected Plant	Percentage Infection (%)	Symptoms
<i>Capsicum annum</i>	5	3	60	Local lesion
<i>Chenopodium quinoa</i>	5	4	80	Local lesion
<i>Nicotiana tabacum</i>	5	2	40	Local lesion
<i>Spinacia oleracea</i>	5	0	-	No

4. Discussion

PVX is one of the most devastating and wide spread viral diseases that cause 15-20% yield losses [1]. Disease incidence and its severity are the important parameter to calculate the disease infection in the field crops as well as for screening purpose to find out the resistance source. Ten varieties were screened out against PVX and observed the disease incidence in the range of 37-60%. Disease severity was recorded according to disease rating scale [9] and varieties were divided to three main groups i.e. moderately resistant, moderately susceptible and Susceptible. FD-74-41, SH 216-A, N 96-25 and FD 8-1 showed the susceptible response, FD 71-1, FSD White, FD 74-50 and KURUDA were moderately susceptible while Tota and FD 76-24 were moderately resistant against PVX. Our results were in accordance with [7]. Moreover [3] showed the similar results and observations. Mechanical transmission showed that *Capsicum annum*, *Chenopodium quinoa* and *Nicotiana tabacum* produced local lesions, while *Spinacia oleracea* did not react against PVX. Therefore, it was concluded that PVX is transmitted mechanically while insect transmission was failed due to unknown reasons. Polyclonal antibodies were used through DAS-ELISA to detect PVX. Mughal et al., [8] performed serological test (ELISA), to detect 8 potato viruses to be prevalent in Pakistan. Similarly [12] used double antibody sandwich-enzyme linked immunosorbent assay to detect simultaneously PVY, PLRV, PVX and PVS in both leaf and sprout samples. Our results were confirmation of work done previously.

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