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

Misbah Iqbal Qamar, Yasir Iftikhar*, Zafar Iqbal, Mustansar Mubeen, Ata-ul-Haq

Department of Plant Pathology, University College of Agriculture, University of Sargodha, Pakistan

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

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 transmitted mechanically. Insect transmission is not known yet. It is immunogenic and easily detected by different serological tests as ELISA and time-resolved fluoroomunoassay [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.

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.

| Grade | Rating Scale | Reaction |
|-------|--------------|----------|
| 0     | No visible symptom | HR       |
| 1     | Mild mottling on the upper leaves | H        |
| 2     | Intervernal 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
3.2. Serological Indexing

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 Insect | Infection (%) | Symptoms  |
|-----------------------|---------------|---------------|-----------|
| *Capsicum annuum*     | 5             | 3             | 60        | Local lesion |
| *Chenopodium quinoa*  | 5             | 4             | 80        | Local lesion |
| *Nicotiana tabacum*   | 5             | 2             | 40        | Local lesion |
| *Spinacia oleracea*   | 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 annuum, 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.

REFERENCES

[1] Abbas. M.F, Hammed. S, Rauf. A, Nosheen. Q, Ghani. A, Qadir. A, and Zakia. S. Incidence of six viruses in potato growing areas of Pakistan, Pakistan Journal of Phytopathology, Vol. 24, No.1, 44-47, 2012.

[2] Adams. M. J. J. F, Antoniw, M. Bar-Joseph, A. A. Brunt, T. Candresse, G. D. Foster, G. P. Martelli, R. G. Milne, and C. M. Fauquet. "Virology Division News: The new plant virus family Flexiviridae and assessment of molecular criteria for species demarcation." Archives of virology, Vol. 149, No. 5, 1045-1060, 2004.

[3] Ahmed. N, Khan. M.A, Khan. N. A, Binyamin. R, and Khan. M. A. Identification of resistance source in potato germplasm against PVX and PVY, Pakistan Journal of Botany, Vol. 43, No.6, 2745-2749, 2011.

[4] İftikhar. Y, Khan. M. A, Rashid. A, Mughal. S. M, Iqbal. Z, Batool. A, Abbas. M, Khan. M. M, Muhammed. S, Jaskani. M.J. Occurrence and distribution of citrus tristeza closterovirus in the Punjab and NWFP, Pakistan, Pakistan Journal of Botany, Vol. 41, 373-380, 2009.

[5] Khalid. S, İftikhar. S, Munir. A, and Ahmad. I. Potato diseases in Pakistan, PARC. Islamabad, pp.165, 2000.

[6] Khalid. S, Mughal. S. M, and Hameed. S. Development of enzyme link immunosorbutant assay for potato virus X in Pakistan, Pakistan Journal of Botany, Vol. 21, No.2, 331-338, 1989.

[7] Mahmood. K. Screening of potato germplasm against PVX and PVY through ELISA and role of phenolic compounds in disease development, M.Sc. (Hons) thesis, Department of Plant Pathology, University of Agriculture, Faisalabad, p. 117-123, 2006.

[8] Mughal. S.M, S. Khalid, T.S. Gillani, and A. Devaux. Detection of potato viruses in Pakistan, In Proceeding 2nd Triennial Conference Kunming China, Pp 189-190, 1988.

[9] Mughal. S.M., and Khan, M.A. Disease rating scale for the assessment of disease severity of PVX and PVY to facilitate the researchers and students working on plant viruses. M.Sc. (Hons.) Agri. Thesis, p. 25. Department of Plant Pathology, University of Agriculture Faisalabad, 2003.

[10] Nosheen. Q, Hameed. S, Mughal. S.M, and Abbas. M.F. Serological identity of Potato virus X (PVX) and PCR characterization of its coat protein (CP) gene, E Science Journal of Plant Pathology, Vol. 02, No.02, 92-96.

[11] Parven. N., Mukhtar. T., Abbas .M.F., and Rauf. C.A. Management of root knot nematode with marigold (Tagetes erecta L.) and antagonistic fungus Paecilomyces lilacinus (Thom) Samson in tomato crop, International Journal of Biology and Biotechnology, Vol.10, No.01, 61-66, 2013.

[12] Román. C. P. D. S, Legorburu. F. J, Pascualena. J, Gil. A. Simultaneous detection of potato viruses Y, leafroll, X and S by DAS-ELISA technique with artificial polyvalent antibodies (APAs), Potato Research, Vol. 31, No. 01, 151-158, 1988.

[13] Sinijarv. R, Jarvekulg. L, Andreeva. E, and Saarma. M. Detection of potato virus X by one incubation europium time-resolved fluorimmmunoassay and ELISA, Journal of General Virology, Vol. 69, 991, 1988.