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IDENTIFICATION OF RESISTANCE SOURCE IN POTATO GERMPLASM AGAINST PVX AND PVY

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Abstract

Potato is attacked by various diseases (bacterial, viral and fungal). PVX and PVY diseases of potato caused by PVX (*Potex virus X)* and PVY (*Poty virus Y)* causes heavy losses to the potato crop. In Pakistan, potato crop losses were reported up to 83% due to viruses. Potato germplasm consisting of 28 genotypes was screened against the PVX and PVY under field conditions during two seasons 2008-10. In case of PVX eight genotypes i.e., 494055-40, FD9619, OCEANIA, FD48-4, TPS-9801, FSD RED, 394021-125, FD3713, showed variable response in both years probably due to their genetic instability and in case of PVY six genotypes i.e., FD1-8, FD9619, CARDINAL, DESIREE, TPS-9802, 393574-61 also showed different response might be due to variation of vector population or due to genetic instability. Out of 28 varieties/lines there were 3 varieties FD3-10, FD3-9 and 393574-61 found to be highly resistant against PVX. Only 3 varieties MIRRATO, ARTERIX and DESIREE were moderately susceptible while 8 were moderately resistant and 6 were found to be resistant against PVX. While in case of PVY out of 28 varieties/lines five varieties TPS-9801, FD32-2, OCEANIA, FD13 and FD3713 were found to be resistant against PVY and four varieties FSD RED, ARTERIX, ORLA and FD3-10 were susceptible. Four varieties were found moderately resistant, nine varieties were found moderately susceptible against PVY. In addition to field screening resistant source of potato was identified through biological and serological test (ELISA test) against PVX and PVY. Screening of different varieties of potato against PVX and PVY diseases to find resistant source to control the disease is an economical way and the results would help in the recognition of available resistant germplasm against the disease, and will be utilized for potato improvement program in Pakistan.

Introduction

Potato (*Solanum tuberosum* L,) is an annual, herbaceous plant belonging to the family Solanaceae. It contains about 79% water, 18% starch, 2% protein, 1% vitamins, minerals and many trace elements. Potato is the world's leading vegetable crop. It is grown in about 140 countries (Haase, 2008).The potato originated from the mountains of South America and in recent year's potato has spread in many countries with warmer and drier climates and it has become important in regions such as the plains of India, Bangladesh, Pakistan, Central America and Argentina etc (Beukema & Eanderzaag, 1990).

PVX causes mild mosaic, interveinal chlorosis and rugosity and sometimes top necrosis. Affected plants produce few tubers which are smaller and under sized. PVX is a latent virus because it can remain latent in the infected plant and therefore sometimes unable to identify on symptom basis. About 20 viruses are known to infect potato. While most important are PVX. PVY, PLRV, PVA, and PVS which cause severe damage to potato crop in Pakistan. Through biological and serological tests (ELISA), 8 potato viruses were detected to be prevalent in Pakistan i-e; PVX, PVY, PLRV, PVS, PVA, PMTV, and PVM (Mughal *et al*., 1988). Degeneration in seed potato occurs when diseased tubers are continuously used as seed source for several years. In Pakistan, potato crop losses were reported up to 83% due to viruses (Mughal & Khalid, 1985). Severe infection can reduce yield by 40-70% (Wustman, 1978). Potato virus X (PVX) infects more than 200 plant species, majority of which belongs to family Solanaceae (Purcifull & Edwardson, 1991).

Losses due PVY in potatoes range between 58-83% in Pakistan (Khalid *et al*., 2000). It induces severe mosaic, rugosity, crinkling, necrosis and affected plants produce small size tubers. Introducing the disease resistant varieties is regarded as a most economical and durable method for controlling plant diseases, especially those caused by viruses. A good deal of research work has been directed towards screening potato germplasm against PVX and PVY to identify resistant sources under adverse environmental conditions and a number of lines resistant to virus were selected that can be used for the future breeding programme.

Material and Methods

For screening purpose 28 varieties/advanced lines of potato of diverse origin/source were planted during two consecutive years in the research area of Department of Plant Pathology. Three replications were made and in each replication plants were selected randomly for screening purpose of potato against PVX and PVY. The distance between P×P was 30cm and between R×R was 60cm. Observations were initiated from 22nd of December 2008 to 9th February 2009 and on similar dates in 2009-10 season, to observe the reaction of varieties/advance lines of potato against PVX and PVY, in several varieties mild to severe mosaic was first observed on young leaves and then rugosity appeared. In order to record the incidence of PVX and PVY following formula was used.

$$\% \text{ Incidence of PVX or PVY} = \frac{\text{No. of infected plants/ unit area}}{\text{Total no. of plants/ unit area}} \times 100$$

Double antibody sandwich ELISA (DAS ELISA) was performed during the 2nd year screening and buffer solutions required for DAS ELISA were prepared by following materials and methods.

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a. Antibody coating buffer: Distilled water I liter, Sodium carbonate (Na2 CO3) C1 0.59 g, Sodium bicarbonate (NaHCO3) 2.93 g. First the chemicals were dissolved in 900 ml distilled water. The pH of the solution was adjusted to 9.6 volume was made up to 1 liter.

b. Virus (antigen) extraction buffer: First phosphate buffer saline (PBS) with 0.05% Tween-20 (PBST) was prepared by dissolving the following salts in 1 liter distilled water. Sodium chloride (NaCl) 40 g, Potassium phosphate (K2PO4) 2 g, Sodium phosphate (Na2PO4) 11.5 g, Potassium chloride (KCl) 2 g, Sodium azide (NaN3) 2 g.These chemicals were dissolved in 800 ml distilled water. Then the volume was made up to the mark of I liter. It is called PBS (stock solution). The stock solution was added by following chemicals: Polyvinyl pyrrolidone (PVP. MW 40000) 2 g, Tween -20 (Polyoxy ethylene sorbitan monolayrate) 0.5 ml/L, Egg ova albumin 2 g, Distilled water 800 ml. Above chemicals were dissolved and volume was made up to 1 liter the pH of the solution was adjusted to 7.4. This extraction buffer was used both for grinding virus samples and making conjugate solution. For grinding virus samples and for making conjugate solution.

c. Washing buffer: 5 x PBS 200ml, Distilled water 800ml, Tween-20 1ml, These were mixed and stirred well and stored at room temperature.

d. Substrate buffer: Diethanol amine 97 ml, Distilled water 800 ml, 97 ml of diethanol amine was added slowly to 800 ml distilled water on an electric stirrer. Concentrated HCl was added drop by drop to adjust pH at 9.8 then the volume was made up to 1 liter.

Leaf samples from 28 varieties/ advanced lines were collected for testing through ELISA against PVX and PVY as described by Clark & Adams (1977). The procedure involved the following steps; the 96-wells of ELISA plate was coated with PVX and PVY each antibody, diluted in coating buffer at 1: 200 and the coated plate was incubated at 40C for overnight. After incubation the plate was washed with PBS-Tween 3 times at 5-minute intervals and these wells were filled with the sap of PVX and PVY infected tissue extracted in extraction buffer and two wells were filled with each of buffer and healthy samples. The plate was incubated for overnight at 40C and washed 3 times with PBST.200µl of enzyme conjugate diluted at 1:200 was added and incubated for overnight at 40C followed by washing as in step 3. 200µl of freshly prepared substrate buffer containing p-nitro phenyl phosphate (75µg / ml) was added to each well. Incubation was done at room temperature for 30 minutes and reaction was visually observed for the development of yellow color. The reaction was stopped by adding 50µl 3M NaOH to each well.

Development of yellow color in the wells indicated the presence of a virus and its intensity which is proportional to the concentration of virus in the plant. Therefore, the positive and negative samples were sorted out by visual observation of yellow color. Reaction was stopped by the addition of 50µl 3M NaOH solution and the plates were photographed.

Results and Discussion

Screening against PVX in 2008-09: During the year 2008-09 twenty eight varieties/advanced lines were screened against PVX. Mild to severe mosaic was first observed on young leaves and then rugosity appeared. The severity of disease increased with the passage of time. The first observation was recorded after 50 days of sowing and 0-75% varieties showed the disease symptom and this percentage increases up to 85% till the last week. In the first week 20 lines were found to be infected with PVX and in 2nd week 21 and goes up to 25 varieties in the last week. Three varieties FD3-10, 393574-61 and FD3-10 were found to be highly resistant and only one variety was found to be highly susceptible i.e., 494055-40, while seven FD3713, KUFRI BADSHA, 394005-115, FSD white, TPS-9813, TPS-9802 and FD13were found to be resistant, 10 varieties/advanced lines were moderately resistant i.e., FD9619, OCEANIA FD1-8, SAFREEN, FD35-25,ORLA, FD49-62, CARDINAL, 39624021, FD32-2. The number of moderately susceptible varieties were five i.e., FD48-4, TPS-9801, MIRRATO, ARTERIX, DESIREE and only two varieties were found to be susceptible FSD RED, 394021-125 (Table 1).

Disease rating scale for PVX and PVY (Mughal & Khan, 2001).

Disease reaction (PVX)		**Severity index disease**
No visible symptoms	0	HR
Mild mottling on the upper leaves.	1	H
Inter venial mosaic symptoms on more than one leaf.	2	MR
Mosaic symptoms on all leaves.	3	MS
Distinct mosaic symptoms on all leaves.	4	S
All above symptoms and small number of small sized tubers	5	HS
Disease reaction (PVY)		**Severity index disease**
No symptoms	0	HR
Blackening and banding of vein on few leaves. Mosaic starting on all leaves.	1	H
Blackening and banding of vein on all leaves, narrowing of leaves, venial necrosis, severe mosaic, leaf crinkling.	2	MR
Rugosity and leaf drop streak, dwarfing	3	MS
Lower leaves dead, drooping collapse of plants with very small tubers	4	S
All leaves dead, stem dead or drying.	5	HS

Table 1. Potato germplasm field screening to PVX during 2008-09.

Resistance level	No. of genotypes	Disease severity	Varieties/lines
HR	3	0	FD3-9, FD3-10, 393574-61
R	7	1	FD3713, KUFRI BADSHA, 394005-115, FSD white, TPS-9813, TPS-9802, FD13.
MR	10	2	FD9619, OCEANIA, FD1-8, SAFREEN, FD35-25, ORLA, FD49-62, CARDINAL, 39624021, FD32-2.
MS	5	3	FD48-4, TPS-9801, MIRRATO, ARTERIX, DESIREE
S	2	4	FSD RED, 394021-125
HS	1	5	494055-40

Screening against PVY in 2008-09: Same 28 varieties/advanced lines were screened against PVY. Germination was completed within a week and aphid started feeding soon after the plants had emerged from the soil continued till maturity of crop. Two varieties FD1-8 and FD9619 were found to be highly resistant and only 2 varieties were found to be highly susceptible i.e., TPS-9802, 393574-61, while 5 varieties TPS-9801,FD32-2, OCEANIA, FD13 and FD3713 were found to be resistant, 5 varieties were moderately resistant i.e., FD3-9, FSD white, CARDINAL, TPS-9813 and 394021-125.The number of moderately susceptible varieties were 10 i.e., MIRRATO, SAFREEN, FD48-4, FD35-25, FD49-62, KUFRI BADSHA, 494055-40, DESIREE, 39624021 and 394005-115 and only four varieties FSD RED, ARTERIX, ORLA and FD3-10 were found to be susceptible (Table 2).

Table 2. Potato germplasm field screening to PVY during 2008-09.

Resistance level	No. of genotypes	Disease severity	Varieties/lines
HR	2	0	FD1-8, FD9619
R	5	1	TPS-9801, FD32-2, OCEANIA, FD13, FD3713
MR	5	2	FD3-9, FSD white, CARDINAL, TPS-9813, 394021-125
MS	10	3	MIRRATO, SAFREEN, FD48-4, FD35-25, FD49-62, KUFRI BADSHA, 494055-40, DESIREE, 39624021, 394005-115
S	4	4	FSD RED, ARTERIX, ORLA, FD3-10
HS	2	5	TPS-9802, 393574-61

Screening against PVX in 2009-10 and ELISA confirmation: During 2nd year 2009-10 in the last week of December all 28 lines/varieties of potato showed infection with PVX, the observations were recorded for 8 weeks to observe the reaction of varieties/advance lines of potato to PVX. Out of 28 varieties/lines there were four varieties FD3-10, FD3-9, FD3713 and 393574-61 found to be highly resistant against PVX, only one 494055-40 was susceptible while twelve varieties were FD1-8,SAFREEN, FD35-25, ORLA, FD49-62, 394021-125, CARDINAL, FD48-4, TPS-9801, 3962402, FD32-2 moderately resistant and eight FD13, Oceania, FD9619, TPS-9802, TPS 9813, Fsd White, 394005-115, Kufri Badshah were found to be resistant against PVX, as similar result were given by Mahmood, (2006) and Thirumalaisamy *et al*., (2003). Qamar (2002) also screened the potato cultivars and they also found high disease incidence. ELISA test was made for the confirmation of PVX from the samples collected from field. 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. Results given in Table 1 and Fig. 1 clearly indicates that there was positive reaction with PVX used as antigen agent with PVX monoclonal antibodies, whereas healthy tissues gave negative reaction and these results clearly revealed that PVX was present in the field samples. Although symptom expression was indicating the incidence of PVX in the field samples and the ELISA test confirmed the virus in the 16 samples. Out of 16 only one showed highly positive reaction while 4 showed mild positive reaction and 11 showed as weak positive reaction. Therefore, keeping in view the weak antigenic property of virus, all samples was tested through ELISA with high concentration of antibodies. These results were similar as the results showed by Rashid & Hassan (2003) who studied in the major potato growing areas of NWFP (Table 3 and Fig. 1).

Table 3. Potato germplasm field screening to PVX and ELISA test during 2009-10.

Resistance level	No. of genotypes	Disease severity	ELISA results	Varieties/lines
HR	4	0	-ve	FD3-9, FD3-10, 393574-61, FD3713
R	8	1	-vs	KUFRI BADSHA, 394005-115, FSD white, TPS-9813, TPS-9802, FD9619, OCEANIA, FD13
MR	11	2	+	FD1-8, SAFREEN, FD35-25, ORLA, FD49-62, 394021-125, CARDINAL, FD48-4, TPS-9801, 39624021, FD32-2
MS	4	3	++	MIRRATO, FSD RED, ARTERIX, DESIREE
S	1	4	+++	494055-40

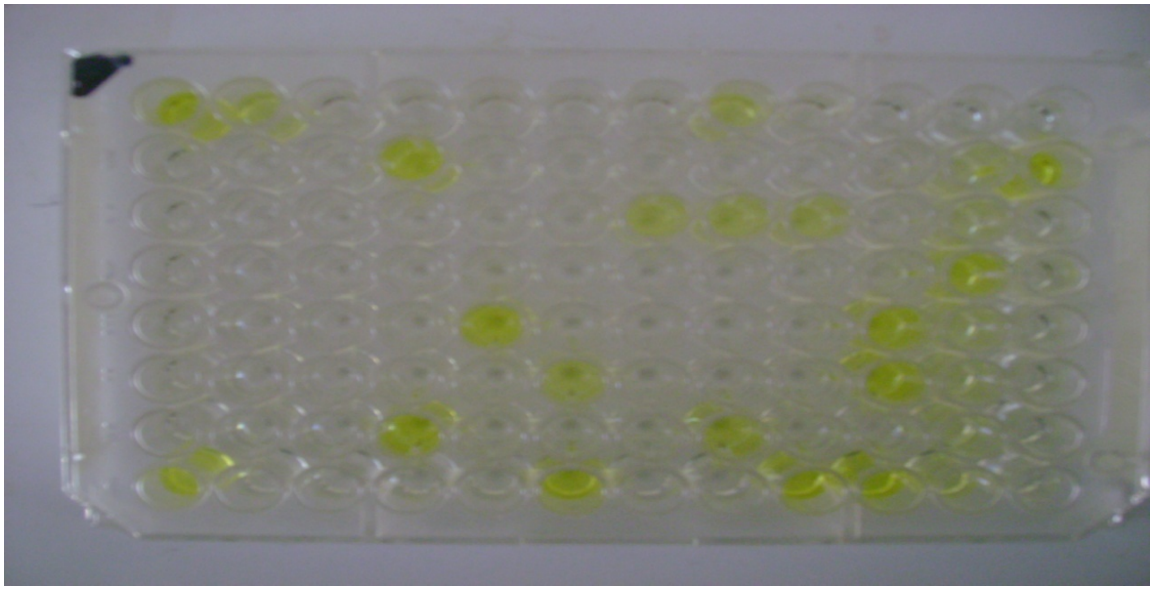

Fig.1. Reaction of PVX and PVY in ELISA plate.

Screening against PVY in 2009-10 and ELISA confirmation: In case of PVY the data was recorded similarly 60 days after planting the tubers and the affected plants showed mosaic, rugosity, crinkling, and necrosis which dominate with the passage of time on the affected plants. Out of 28 varieties/lines 6 varieties FD1-8, TPS-9801, Oceania, FD13,, FD3713 and FD32-2 were found to be resistant against PVY, 5 varieties FD3-9, FSD white, TPS-9813, FD9619, 394021-125 were found moderately resistant and 10 varieties MIRRATO, SAFREEN, FD48-4, FD35-25, FD49-62, CARDINAL, KUFRI BADSHA, 494055-40, 39624021, 394005-115, were found moderately susceptible while 7 FSD RED, Orala, FD3-10, Arterix, Desiree, TPS-9802 and 393574-61 were susceptible against PVY (Table 4).. Jan & Khan (1995) reported that potato virus X (PVX), potato virus Y (PVY) infecting commercially grown potatoes in the upper Kaghan valley of Pakistan; however, PVX was less common and PVY was more and those same results comes in our study.

Leaf samples from 28 potato varieties/advanced lines with and without symptoms or suspected symptoms were collected from field and subjected to ELISA test. The results obtained were assessed visually. ELISA test was made for the confirmation of PVY from the samples collected from field. Results (Table 4 and Fig. 1) clearly indicate that there was positive reaction with PVY used as antigen agent with PVY monoclonal antibodies, whereas healthy tissues gave negative reaction and these results clearly revealed that PVY was present in the field samples. The severity of symptoms in naturally infected potato plants compared very well with color development in ELISA plate. Although symptom expression was indicating the incidence of PVY in the field samples but ELISA test confirmed the virus in the 8 samples. Out of 28, 6 samples showed negative reaction while 7 showed highly positive reaction while 10 showed mild positive reaction and 5 showed as weak positive reaction against PVY. Jarjees (2000) also used the ELISA for rapid detection of PVY in Iraq and obtained significant results. Abou- Jawdah (2001) studied potato fields in the 2 main production areas of Lebanon, the Bekaa & Akkar plains, for viruses and other pathogens of significance for a potato seed certification programme. Positive reaction was observed with PVY 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 (Clark & Adams, 1977). It provided rapid, reliable and accurate diagnosis of PVY. The severity of symptoms in naturally infected potato plants compared very well with color development in ELISA plate. Although symptom expression was indicating the incidence of PVY in the field samples but ELISA test also confirmed the virus in the samples.

Table 4. Potato germplasm field screening to PVY and ELISA test during 2009-10.

Resistance level	No. of genotypes	Disease severity	ELISA results	Varieties/lines
R	6	1	-ve	FD1-8, FD32-2, TPS-9801, OCEANIA, FD13, FD3713
MR	5	2	+	FD3-9, FSD white, TPS-9813, FD9619, 394021-125
MS	10	3	++	MIRRATO, SAFREEN, FD48-4, FD35-25, FD49-62, CARDINAL, KUFRI BADSHA, 494055-40, 39624021, 394005-115
S	7	4	+++	DESIREE, FSD RED, ARTERIX, ORLA,FD3-10, TPS-9802, 393574-61

PVX and PVY may consist of some variants which need to be detected, differentiated and characterized on the biological, serological and molecular bases to strength the breeding programs. Genetic variability in potato germplasm for PVX and PVY seems to be narrow. It would be desirable to broaden this base through breeding and biotechnology for which collection and use of local germplasm of potato can play a greater role. The change of genotype response against PVX and PVY during the both years might be attributed to various causes like genetic makeup of plant and several other biotic and abiotic factors. The screening results revealed that some environmental factors and most importantly genetic makeup must be involved in conditioning resistance and susceptibility which need to be investigated and studied in detail. The genotypes screened during this study should be used for further studies for searching resistance sources under different field conditions and artificial inoculation for genetic manipulations and breeding purpose. The most important problem comes during screening is that some genotypes appear to be susceptible at one place but they turns out to be resistant at another place, because of that environmental factors affect on disease progress should also be studied for proper evaluation of germplasm against different diseases. Screening of potato germplasm both local and exotic needs to be continued. Till such times, resistant sources/cultivars become available, it is necessary to reduce severe incidences of PVX and PVY through various management practices.

Conclusion

Some genotypes showed variable responses against PVX, and some showed variable responses against PVY in the both seasons might be due to genetic instability. So screening of potato germplasm is necessary for identification of resistant source.

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