ELISA-BASED SCREENING OF POTATO GERMPLASM AGAINST POTATO LEAF ROLL VIRUS*

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ABSTRACT

In a study conducted in Plant Pathology Department, University of Agriculture, Faisalabad, Pakistan during 2006, 29 potato varieties/lines were screened against potato leaf roll virus (PLRV) under natural field conditions favourable to induce maximum virus infection of PLRV. Initially, the disease rating was based on the symptomatology. On the basis of symptomatology only one line i.e., 394032-16 was found free of symptoms while SH-5, FD 1-8 and FD 1-9 were considered as moderately resistant. Nineteen varieties/lines were considered as moderately susceptible and six were susceptible to PLRV. ELISA test was carried out to identify resistant source and confirm the presence of virus. The results showed that only 25 varieties /lines were ELISA positive. This proves that ELISA is more reliable and sensitive serological assay to detect the resistant virus free source.

KEYWORDS: ***Solanum tuberosum;*** **genotypes; plant viruses; elisa technique; Pakistan.**

INTRODUCTION

Potato (*Solanum tuberosum* L.) belonging to the family *Solanaceae,* is the world's leading food and vegetable crop which originated in the Andean region of Peru. In Pakistan, potatoes are consumed as the most important vegetable. Its produce comes from three crops during a year; autumn and spring crops in the plains and summer crop in the hilly areas. In Pakistan, average yield of potato is 17652 kg per hectare, which is very low as compared to other countries (4). Many factors including diseases are responsible for low potato yield. Among these, prevalence of viral diseases like potato leaf roll virus (PLRV), potato virus Y (PVY) and potato virus X

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(PVX) are the most common, serious and widely distributed. A high incidence of PLRV (48-72%) was observed by Hassan *et al.* (7) in NWFP (now Khyber Pakhtunkhwa).

Arif *et al*. (5) studied biological, physical and serological properties of PLRV in Pakistan. The mean incidence of PLRV in the plains was recorded as 44 percent in general, 53 percent in the market or uncertified seed and 11 percent in certified seed. Ali *et al*. (3) surveyed the incidence of six potato viruses (PLRV, PVX, PVY, PVS, PVA and PVM) in spring, summer and autumn crops of NWFP. A total of 1338 samples from 76 fields were tested by dot-immunobinding assay. Two major aphid-borne viruses, PLRV and PVY were frequently detected in potato with incidence ranging from 0-14.7 percent in spring crops, 1.8-45.5 percent in summer crops and 0-71 percent in autumn crop. Three potato growing areas of NWFP (Pabbi, Bunair and Swabi) were surveyed (10) to determine the incidence of potato viruses in autumn crop. PLRV, PVY, PVX and PVS occurred predominately and were identified on the basis of symptomatology, host range, serology and transmission properties. The mean incidence of PLRV, PVY, PVX, PVS and PVA was recorded as 4.29, 5.97, 13.26, 36.19 and 0.47 percent, respectively. The presence of PLRV, PVX, and PVY was confirmed in another study (9) in 16 samples by DAS-ELISA. All samples were free from PLRV, although potato varieties like Desiree showed the leaf roll symptoms as its varietal character.

PLRV is relatively more serious and occurs throughout the country being more serious in plains of Punjab and Sindh. Its incidence has been recorded between 20-60 percent depending upon source of seed, virus strain, vector population and weather conditions. Both primary and secondary infections of PLRV are encountered in field. Plants infected during the current year developed symptoms on the upper leaves, while in case of previous year, infected leaves became stiff at the base, thickened and rolled inward. The infected plants are yellowed and sometime dwarfed.

Under natural conditions, in case of current year infection, PLRV entirely transmitted by many aphids in a persistent, circulative and non-propagative manner. Of these green peach aphid, *Myzus persicae* is the most efficient and economically important. This aphid survives on hundreds of host plants (over 40 plant families). Broad leaved weeds can be very suitable host plants for green peach aphid i.e. *Convolvulus arvensis* L. (Lehli) and *Euphorbia helioscopia* L (Chatridodhak). Previous year infection mainly

originates from seed tubers. Use of resistant variety/varieties is one of the durable and economical methods of disease control.
In present study potato varieties/lines were evaluated under natural field conditions conducive to PLRV and resistant source was identified on the basis of disease severity index and ELISA.

MATERIALS AND METHODS

Screening of potato germplasm

A disease-screening nursery consisting of 29 varieties/advanced lines of potato was established in the field of Plant Pathology Department, University of Agriculture, Faisalabad, Pakistan during 2006. Nineteen potato varieties/advanced lines were obtained from National Agricultural Research Centre, Islamabad and ten from Ayub Agricultural Research Institute, Faisalabad. These were planted in a completely randomized block design. Twenty tubers of each variety/line were planted in the rows, replicated thrice keeping plant to plant and row to row spacing of 30 and 60 cm, respectively. Fallow field was well prepared and farmyard manure was added @ 20 tons per hectare. Fertilizer application (2:1:1) was consisted of 250-kg N (in 3 splits), 125-kg P and 125-kg K per hectare. Irrigation was applied at 15 days intervals and stopped 15 days before harvesting. All conventional agronomic practices such as sowing (mid September to mid October), earthing up, weeding, etc. were adopted to keep the crop in a sound growing condition except spraying.

Symptomatology

Symptoms were recorded depending upon current year infection when the upper leaves were slightly curled and sometimes showed yellow or purple pigmentation whereas in case of previous year (2005) infection, lower leaves were stunted with tuber necrosis. During studies, current year infection was observed and disease progression was assessed through rating scale (8).

Serological test

ELISA test was carried out to detect the virus infection from the samples (6). ELISA plate was coated with 200µl PLRV monoclonal antibodies in each well. The coated plate was incubated at 4°C for 24 hours followed by thorough washing with PBST (1x) three times at 5 minutes intervals. Fresh leaf samples were collected from the plants showing characteristic

symptoms of PLRV. Leaves were chopped into small pieces and ground in pestle and mortar in phosphate buffer. Sap was filtered through double-layered muslin cloth.

Each plate was charged with 200µl the antigen (sap of PLRV infected tissue) extracted in extraction buffer with micropipette. Buffer and healthy samples were also charged as a control. The plate was incubated for 24 hours at 4°C followed by washing. 200µl of enzyme conjugate diluted at 1:200 was added in each well and incubated for 24 hours at 4°C. After washing freshly prepared substrate buffer containing p-nitro phenyl phosphate (1mg/ml) was added to each well at the rate of 200µl. Incubation was done at room temperature (25ºC) for 30 minutes and reaction was visually observed for the development of yellow colour and also read in ELISA reader (model Biotek-L800) at 405 nm. The reaction was stopped by adding 50µl 3M NaOH to each well. Potato varieties were classified for resistance against PLRV according to Khan *et al.* (8).

Symptoms	Disease severity index	Reaction grade
No visible symptoms	0	Immune
Rolling of leaves in case of primary infection and lower leaves in case of secondary infection, erect growth	1	Resistant
Rolling of leaves extending, leaves become stiff and leathery, stunting of plants and erect growth	2	Moderately resistant
Short internodes, papery sound of leathery leaves, rolling and stunting of whole plants. Young buds are slightly yellowish and purplish	3	Moderately susceptible
Clear rolling of leaves, severe stunting, few tubers and tuber necrosis	4	Susceptible

RESULTS AND DISCUSSION

Out of 29 varieties/lines tested, only one line 394032-16 was found to be free of symptoms of PLRV and thus showed immunity against potato leaf roll virus (Table 1). FD1-9, SH-5 and FD1-8 exhibited moderately resistant response to PLRV, while 19 varieties were moderately susceptible and six varieties were susceptible to PLRV. These findings are in conformity with earlier work (8) where 15 potato varieties/lines were screened against PLRV infection developing from natural inoculum under field conditions but no resistance in commercial varieties (FSD-Red, Desiree, Jose B, Dura, Cardinal, Sante, SH-5, TPS-9808 and TPS-9801) was found. Similar studies were also conducted earlier (2) where no resistant source was reported through ELISA tests.

Leaf samples of germplasm under investigation were indexed through ELISA to confirm PLRV infection. There was positive reaction only in 25 samples i.e. 9803, Diamont, 9802, 391202-158, 396266-33, 394005-115, 396240-181, Cardinal, Desiree, 394021-120, 9801, 9808, 369240-21, 394028-37, TPS-9813, 9814, 394055-44, FD48-1, FSD-White, FD3-15, FD48-41, FD35-36,

Table 1. Reaction of potato varieties/lines to PLRV.

S.No.	Variety/line	Disease severity index	Level of resistance	ELISA reaction OD at 405nm
1.	9803	4	S	0.151 +ve
2.	Diamont	3	MS	0.082 +ve
3.	394032-16	0	I	0.029 -ve
4.	9802	4	S	0.073 +ve
5.	391202-158	3	MS	0.134 +ve
6.	396266-33	3	MS	0.080 +ve
7.	394005-115	3	MS	0.079 +ve
8.	396240-181	3	MS	0.061+ve
9.	Cardinal	3	MS	0.063 +ve
10.	Desiree	3	MS	0.069 +ve
11.	394021-120	3	MS	0.083 +ve
12.	9801	3	MS	0.085 +ve
13.	9808	3	MS	0.084 +ve
14.	SH-5	3	MR	0.030 -ve
15.	396240-21	4	S	0.146 +ve
16.	394028-37	4	S	0.141 +ve
17.	TPS-9813	3	MS	0.075 +ve
18.	9814	3	MS	0.070 +ve
19.	394055-40	3	MS	0.132 +ve
20.	FD48-1	3	MS	0.063 +ve
21.	FSD-White	4	S	0.137 +ve
22.	FD3-15	4	S	0.146 +ve
23.	FD1-9	2	MR	0.044 -ve
24.	FD48-41	3	MS	0.185 +ve
25.	FD1-8	3	MR	0.034 -ve
26.	FD35-36	3	MS	0.091 +ve
27.	FD3-9	3	MS	0.098 +ve
28.	FD49-28	3	MS	0.081 +ve
29.	393574-61	3	MS	0.071 +ve

FD3-9, FD-49-28 and 3935741-61 with PLRV used as antigen against PLRV monoclonal antibodies, whereas healthy tissues and buffer were ELISA negative (Table 2). Moderate yellow colour was observed in these samples which confirmed the presence of PLRV and absorbance was measured at 405nm.

On the basis of symptomatology all potato varieties manifested variable incidence of PLRV except 394032-16 in the field but ELISA test confirmed the presence of virus only in 25 samples. Although, symptomatology is the initial step to diagnose the disease in field and to categorize varieties, yet it is not a reliable criterion because symptoms development is influenced by many factors such as environmental conditions, insect sucking, nutritional deficiency, growth stage, time of infection, host genotype, virus strains, etc.

Table 2. Absorbance of potato samples in ELISA plate at 405 nm.

	Total number of wells charged	Number of wells with positive reaction	Colour	OD values at 405 nm
PLRV infected tissue	29	25	Moderate yellow	0.061-0.185
Healthy	4	-	Negative	0.021
Buffer	4	-	Negative	0.034

On the other hand ELISA tests are more reliable for virus detection and identification of resistant source. This is the reason that ELISA confirmed the PLRV only in 25 field samples. So it can be concluded, that ELISA results depend upon low virus titre or antigenic property of the tested virus. The method for collection of samples can also affect the ELISA reactivity. Similar findings were reported by Mughal *et al.* (11) who conducted the biological and serological tests; particularly the ELISA enabled the detection of 8 potato viruses from Pakistan including PLRV. They also confirmed PLRV through differential hosts. Ahmed *et al.* (2) surveyed seven main potato growing districts of Punjab during autumn season and reported PLRV, PVX and PVY. From 169 fields, 1227 leaf samples were collected and assayed by ELISA. PLRV was detected from 6.54 percent samples, PVX from 13.18 percent and PVY from 23.06 percent samples. Maximum PLRV incidence was detected from Jhang (12.82%), followed by Sialkot (11.34%), Toba Tek Singh (5.55%), Gujranwala (5%), Kasur (4.41%), Okara (3.63%) and Sahiwal (3.03%).

The results clearly showed that PLRV infects almost all potato varieties/lines grown in the country. Therefore, extensive screening of potato germplasm on serological and biological indexing is required to establish a resistance source against PLRV.

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