Research Article

Field Evaluation and Serological Detection of Potato Leaf Roll Virus in Potato Germplasm

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ABSTRACT

Potato tuber (*Solanum tuberosum* L.), a main staple food ranked 4th in the world followed by rice, wheat and maize. Potato leaf roll virus (PLRV) is the most vital pathogen of potato in Pakistan that cause 90% losses and transmitted by green peach aphid (*Myzus persicae*).Twenty cultivars of potato were screened against PLRV. Twenty samples were collected on the basis of symptoms and tested by serological assay (DAS-ELISA). Disease incidence was calculated using prescribed formulae. Among all tested cultivars, ten cultivars were found resistant, five cultivars moderately resistant, one cultivar moderately susceptible and two cultivars highly susceptible. Detection of PLRV was done by DAS-ELISA which indicated that three cultivars found resistant, eleven moderately resistant, two moderately susceptible and four cultivars were susceptible.
Key words: PLRV, Cultivars, ELISA, Screening, Resistance, Management.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the world's leading staple vegetable food crop and ranked fourth in production after rice, wheat and maize.[1] The potato tuber is an excellent source of carbohydrates, proteins and vitamins.[2] Potato is cultivated on an area of 176.2 thousand hectares with annual production of 4134.6 thousand tons in Pakistan.[3]

A significantly high number of pathogens from one generation to the next are propagated vegetative material. Among stall strains, almost thirty-seven species of virus can infect potato crop naturally. In Pakistan potato is cultivated three times in a year like autumn, spring and summer crop in hilly and plains areas. High yielding foreign potato varieties significantly increased the yield of potato crop in Pakistan but at the same time new viral problems like PVX, PVY, PVS, PLRV, PVA and PVM have been reported in spring, summer and autumn potato crop of Pakistan and cause up to 83% yield losses.[4] ELISA test is used for the detection of mostly viruses.[5]

PLRV is known to be devastating pathogen among all other potato viruses to yield of crop.[6] PLRV belongs to genus *Polero virus* and have family *Luteoviridae*.[7] The virus has isometric particles, having diameter 24nm.[8] This virus has positive sense Ss RNA of about genome is 5.9 kb.[9] Many species of insects are responsible for the PLRV transmission, among these green peach aphid (*Myzus persicae*) is most efficient vector which transmit PLRV and first reported in Pakistan during 1978.[10] In Pakistan, PLRV has been an evolving problem and present in all potato growing areas.[11]

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Keeping in view the status of potato and its viral problem, the current study was planned to evaluate the available potato cultivars against PLRV under field conditions by using serological test (DAS-ELISA).

MATERIALS AND METHODS

Collection of planting material

Twenty potato cultivars viz. SL15-10, FD63-1, FD78-36, Sante, FD74-21, SLM5-2, FD76-18, FD61-3, SL15-10, SL14-15, Simply Red, FD35-36, FD73-73, FD78-51, SL13-43,FD76-67, FD71-1, SL9-14,FD77-4 and Cardinal were obtained from Plant Virology Section, AARI (Ayub Agricultural Research Institute), Faisalabad and PRI (Potato Research Institute) Sahiwal, Pakistan.

Establishment of potato germplasm under field conditions

A disease-screening nursery consisting of twenty cultivars of potato was established in the field of Plant Virology Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan during 2015-16. These were planted in a randomized complete block design with three replications. Tubers of each cultivar were grown in the field by maintaining P×P distance of 30 cm and R×R distances of 60 cm respectively. Fertilizer application (2:1:1) was consisted of 250-kgN (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 practices such as sowing (mid-September to mid-October), earthing up and weeding were adopted to keep the crop in a sound growing condition except spraying.

Sampling

During the Year 2015-16, sampling of potato leaves was conducted. A total of 20 samples of 8-10 weeks old field growing potato plants were collected on the basis of virus and viral like symptoms. A single sample was consisted of three single leaflets taken from top, middle, bottom and placed in polythene bag. Samples were appropriately labelled to indicate location, sample number, sample collector name, sampling depth and nature of sample (soil sample or tertiary/fibrous roots with soil) and date of collection. These samples were stored same day at 4°C in plant virology lab until further processing.

Data recording

The disease incidence was recorded at the base of visual symptoms of every line. Incidence %age was calculated by following formula;

$$\text{Disease incidence (\%)} = \frac{\text{Infected Plants}}{\text{Total healthy plant}} \times 100$$ [12]

and find the level of resistance and susceptibility of potato cultivars on the basis of the scale.[13]

Serological Assay

DAS-ELESA is used for the testing of PLRV from collected samples from field.[14] Protocol is given below; 96- wells of ELISA plate were coated with PLRV antibodies, each diluted in coating buffer at 1:200. The coating plate was incubated at 4°C for overnight. After Incubation the plate was washed with PBS-Tween 3 times after 5 min intervals. These wells were filled with the sap of PLRV infected tissue extracted in extraction buffer. Three and four wells were filled with each of buffer and healthy samples, respectively. The plate was incubated for overnight at 4°C and washed 3 times with PBST. 200µL of enzyme conjugate diluted at 1:200 was added and incubated for overnight at 4°C 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. The reaction strength was rate visually as (- = no reaction, += weak reaction, ++ =definite reaction, +++ =very strong reaction). Incubation was done at room temperature for 30 min and reaction was visually observed for the development of yellow colour. The reaction was stopped by adding 50µL of 3M NaOH to each wall.

For the confirmation of ELISA results, disease incidence data was observed as per internationally accepted disease rating scale (Table 1) for PLRV.[15]

RESULTS

Current research was conducted to assess the available potato cultivars against PLRV. Leaf rolling (mid to progressive) was recorded after seventy days of crop sowing. Mixed response was observed in these cultivars. Nine cultivars (SL15-10, FD63-1, FD78-36, Sante, FD74-21, SL5-2, FD76-18, FD61-3, SL14-15) were found resistant, five cultivars (Simply Red, FD35-36, FD73-73, FD78-51, SL13-43) were moderately resistant, two cultivars (FD71-1, SL9-14) were moderately susceptible, one cultivar FD71-1 is susceptible and two cultivars (FD77-4, Cardinal) were highly susceptible under field conditions during 2016 (Table 2, Figure 1).

The definite response of all cultivars was proved by the process of ELISA. Resistant varieties showed negative reaction with no colour development in micro titer plate. The wells containing moderately resistant genotypes depicted light yellow colour that is denoted by + sign. The reaction of moderately susceptible varieties were denoted by ++ showing the moderately yellow colour. ELISA results of susceptible and highly susceptible

Table 1: Disease rating scale for PLRV.

Disease scale	Disease incidence %	Symptoms	Reaction group
0	0	No symptoms	HR*
1	1-20	Rolling of upper leaves (Primary infection)	R
2	21-30	Rolling of upper and lower leaves (Secondary infection), erect growth.	MR
3	31-40	Rolling of leaves extending, leaves become stiff and leathery, stunting of plants and erect growth.	MS
4	41-50	Short internodes, papery sound of leathery leaves, rolling and stunting of whole plants. Young buds are slightly yellowish and purplish.	S
5	51-100	Clear rolling of leaves, sever stunting, few tubers and tuber necrosis	HS

*HR = Highly resistant, R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S= Susceptible, HS = Highly susceptible

Table 2: Response of potato germplasm against PLRV in field conditions.

Cultivar #	Cultivars	Rating	Response
1	Simply Red	2	MR*
2	FD71-1	4	S
3	FD77-4	5	HS
4	FD63-1	1	R
5	FD78-36	1	R
6	FD76-67	3	MS
7	Sante	1	R
8	FD74-21	1	R
9	FD35-36	2	MR
10	SL5-2	1	R
11	FD76-18	1	R
12	FD61-3	1	R
13	SL9-14	4	MS
14	FD73-73	2	MR
15	FD78-51	2	MR
16	SL15-10	1	R
17	SL14-15	1	R
18	SL13-43	2	MR
19	Cardinal	5	HS

LSD =2.024
*HR = Highly resistant, R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S= Susceptible, HS = Highly susceptible

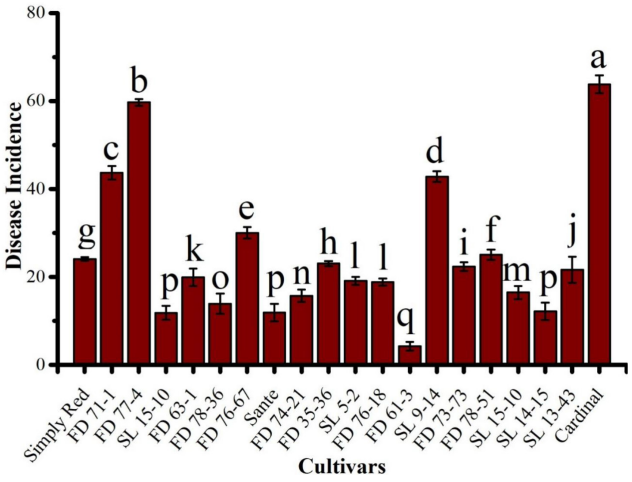

Figure 1: Response of potato germplasm against potato leaf roll virus in field.

cultivars were strong and highly strong which were +++ and ++++, respectively. The results of serological assay were further explained after obtaining the mathematical values through ELISA reader (Table 3). Optical density values (OD) were recorded at 405 nm which depicted maximum values for highly susceptible varieties and minimum values for resistant varieties. The OD values of resistant varieties range from (0.278-0.498), moderately resistant (0.876-0.988), moderately susceptible (1.365-1.564), susceptible (2.254) and highly susceptible (2.262-2.367).

DISCUSSION

In current study, nine cultivars were resistant, five cultivars showed moderately resistant response against PLRV, two cultivars fall under the category of moderately susceptible and highly susceptible each and only one genotype was susceptible. These results are contrary to the findings of a researcher who tested 15 cultivars against PLRV and found no resistant varieties during field experiment.[16] The genetic potential of such varieties can be exploited by the minimum application of chemicals against potentially destructive pathogens. The resistance to infection in the field is not necessarily linked with the resistance to PLRV multiplication and accumulation. In the field, plant to plant spread of virus in resistance cultivars is less because of lower virus titer[17-20] tested thirty-six potato cultivars in the field for resistance to potato leafroll virus (PLRV). One hundred and forty-eight potato clones/germplasm were screened at Murree and Faisalabad for the detection of potato leafroll virus (PLRV), by using enzyme linked immune sorbent assay (ELISA). The potato leaf samples collected from Murree were found to be

Table 3: Detection of PLRV through serological test (DAS-ELISA).

Cultivar #	Cultivar	ELISA	Level of Resistance/ Susceptibility	OD Value at 405nm
1	Simply Red	+*	MR**	0.889
2	FD71-1	+++	S	2.254
3	FD77-4	++++	HS	2.262
4	FD63-1	--	R	0.299
5	FD78-36	--	R	0.278
6	FD76-67	++	MS	1.564
7	Sante	--	R	0.301
8	FD74-21	--	R	0.479
9	FD35-36	+	MR	0.988
10	SL5-2	--	R	0.397
11	FD76-18	--	R	0.289
12	FD61-3	--	R	0.386
13	SL9-14	++	MS	1.365
14	FD73-73	+	MR	0.876
15	FD78-51	+	MR	0.954
16	SL15-10	--	R	0.398
17	SL14-15	--	R	0.498
18	SL13-43	+	MR	0.977
19	Cardinal	++++	HS	2.367
21	+ve control			2.256
22	+ve control			2.253
23	-ve control			0.976
24	-ve control			0.954

*Dark yellow= Very strong (++++), Yellow=Strong (+++)=Susceptible, Moderate yellow =Moderate (++) Moderately Susceptible, Light yellow=Light (+)= Moderate Resistance, No Color= Free (-) = Resistant

***HR = Highly resistant, R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S= Susceptible, HS = Highly susceptible

infected with PLRV at the rate of 3.7% in the material produced through local crosses and 28.56% in the imported material. The potato genotypes for resistance to PLRV are usually evaluated in field exposure trials in which PLRV disease incidence in advanced lines is compared with standard cultivars. Most of the varieties/lines were moderately resistant and moderately susceptible. The moderately susceptible to moderately resistant response of majority of potato varieties had already been reported.[21] Such type of varieties/lines exhibiting tolerant responses were generally high yielding and might be a good source for the vegetable breeders to produce virus free seed through tissue culture techniques. In field exposure trials, some potato genotypes were susceptible to PLRV infection but resistance to virus accumulation, where as other potato genotypes might resist to infection but susceptible to virus accumulation.[22,23] Therefore the combination of both types of resistance would protect the potato crop from PLRV infection and its spread could be minimized.[24,25] The contradictory results between different studies may be due to the fact that the later the plant becomes infected, the less time there is available and the less conducive the conditions are for virus replication, accumulation and systemic translocation inside the plant.[26] Temperature variation at different experimental sites may also affect the plant virus interaction which may result in varied response during screening.[27]

Despite, symptomatology is the basic method to identify and diagnose the disease in field, yet it is not a reliable criterion because symptoms development may be affected by environmental factors, insect attack, nutrient toxicity or deficiency, growth stage, time of infection, host cultivar, virus strains, etc. ELISA is a reliable method for detection and identification virus. In present study, field screening based upon symptomology was confirmed by using ELISA that depicted strong reaction for highly susceptible and susceptible varieties, moderate reactions for moderately susceptible and moderately resistant varieties and weak or no reaction for resistant genotypes. Our results are conformity with those of [28] who detected eight potato viruses from Pakistan by utilizing ELISA techniques. Another study was conducted in which different regions of Punjab province were surveyed for the sample collection of symptomatic potato leaves. Overall 1227 samples from 169 fields and detected PLRV. PVX and PVY were also detected from diseased samples through ELISA techniques and maximum disease incidence of PLRV was recorded.[29]

CONCLUSION

The present study revealed that out of twenty; nine cultivars described above were resistant, five genotypes were moderately resistant, two showed moderately susceptible, one was susceptible while FD77-4 and cardinal were highly susceptible. Morphological screening were confirmed through serological assay. It can be concluded that resistant varieties strongly be recommended for cultivation. All the cultivars should be subjected to further studies regarding growth and yield parameters in order to find out their potential to adapt the varying environmental conditions. Epidemiological studies with spatial and temporal distribution of the disease would provide a strong base for an ultimate decision regarding discarding or further use of a cultivar in a breeding programmes.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

ABBREVIATIONS USED

PLRV: Potato leafroll Virus; **ELISA:** Enzyme Linked Immuno Sorbent Assay; **DAS:** Double Antibody Sandwich; **PVX:** Potato Virus X; **PVY:** Potato Virus Y; **PVA:** Potato Virus A; **PVM:** Potato Virus M.

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