Field Evaluation and Serological (DAS-Elisa) Detection of Potato Leaf Roll Virus in Potato Germplasm

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Abstract: Potato tuber (*Solanum tuberosum* L.) is fourth largest staple food in world followed by wheat, rice and maize. In Pakistan, Potato leaf roll virus (PLRV) is one of the most important diseases of potato. It is transmitted by green peach aphid (*Myzus persicae*) and causes 90% losses. Screening trial of twenty cultivars of potato was established against PLRV. Among twenty cultivars, ten cultivars (SL15-10, FD63-1, FD78-36, Sante, FD74-21, SL5-2, FD76-18, FD61-3, SL15-10, SL14-15) were found resistant, five cultivars (Simply Red, FD35-36, FD73-73, FD78-51, SL13-43) were moderately resistant, one cultivar (FD76-67) was moderately Susceptible, two cultivars (FD71-1, SL9-14) were moderately susceptible and two cultivars (FD77-4, Cardinal) were highly susceptible. Serological test (DAS-ELISA) was performed for the detection of PLRV and detected the virus in three cultivars (SL10, FD63-1, Sante) were found resistant, eleven cultivars (SL5-2, FD76-18, FD61-3, SL15-10, SL14-15, FD78-36, Simply Red, FD35-36, FD73-73, FD78-51, SL13-43) were moderately resistant, two cultivars (FD76-67, FD78-51) were moderately susceptible, two (FD71-1, SL9-14) were moderately susceptible and four cultivars (FD71-1, FD77-4, SL9-14, Cardinal) were susceptible.

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Introduction:

The cultivated potato (*Solanum tuberosum* L.) is the world's leading staple vegetable food crop and ranked fourth in production after rice, wheat and maize (Rauscher *et al*., 2006). The potato tuber is an excellent source of carbohydrates, proteins and vitamins (Ahmad *et al*., 2011). In Pakistan, potato is cultivated over an area of 176.2 thousand hectares with an annual production of 4134.6 thousand tons (GOP, 2015-16).

A significantly high number of pests and pathogens can be carried over from one generation to the next by propagated vegetative material. Among them, at least 37 viruses can naturally infect potato crops. Potato is cultivated three times during a year; autumn, spring and summer crops in the plains and the hilly areas of Pakistan. 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 upto 83% yield losses (Mughal and Khalid, 1986). Most viruses can effectively be detected by ELISA tests (Petrunak *et al*., 1991).

Keeping in view the importance of potato crop 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 (Ayyub Agricultural Research institute), Faisalabad and 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. completely randomized block design. Tubers of each cultivar were grown in the field by maintaining plant to plant distance of 30cm and row to row distances of 60cm 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), earthening 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 and bottom and placed in polythene bag. Samples were appropriately labelled to indicate location, sample number, sample collector name, GPS coordinates, sampling depth, nature of sample (soil sample or tertiary/fibrous roots with soil) and date of collection. These samples stored 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;

$$\text{Disease incidence (\%)} = \frac{\text{Infected plants}}{\text{Total plant}} \times 100$$

(Ali *et al*., 2010) And find the level of resistance and susceptibility of potato cultivars on the basis of the following rating scale by Khan *et al*., (2006).

Serological Assay:

The samples from the field were tested for PLRV by double antibody sandwich ELISA (DAS-ELISA) as described by Clark and Adams, (1977). Procedure involved the following steps; 96- wells of ELISA plate were coated with PLRV antibodies, each diluted in coating buffer at 1:200. The coating plate was incubated at 2°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. 200µLof 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 50µLof 3M NaOH to each wall.

In confirmation of the ELISA results, disease incidence data was observed as per internationally accepted disease rating scale (Table 1) for PLRV (Mughal & Khan, 2001).

Result And Discussion:

Mild to progressive leaf rolling was observed after 70 days of sowing. Mixed response was observed in these cultivars. Ten cultivars (SL15-10, FD63-1, FD78-36, Sante, FD74-21, SL5-2, FD76-18, FD61-3, SL15-10, 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 and two cultivars (FD77-4, Cardinal) were highly susceptible under field conditions during….?? (Table 2, Figure 1).

Three cultivars (SL15-10, FD63-1, Sante) were found resistant, eleven cultivars (SL5-2, FD76-18, FD61-3, SL15-10, SL14-15, FD78-36, Simply Red, FD35-36, FD73-73, FD78-51, SL13-43) were moderately resistant, cultivars (FD76-67, FD78-51, FD71-1, SL9-14) were moderately susceptible and four cultivars (FD71-1, FD77-4, SL9-14, Cardinal) were susceptible (Table 3).

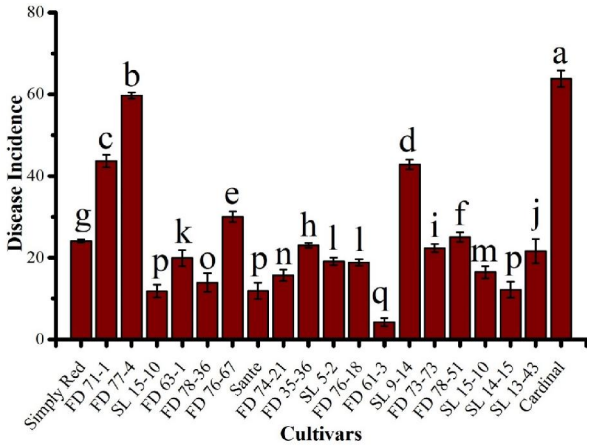

Figure 1. Response of potato germplasm against potato leaf roll virus in field

Seven varieties/lines Astrix, Mirrato, Oceania, Orla, Hermes, Safreen and 396266-33 were found to be highly resistant against PLRV. Four entries FD 7-2, 394021-120, FD 48-4 and FD 49-62 appeared to be resistant. Phytosanitary measures can also be used to reduce initial virus inoculum. 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 (Barker and Woodford 1992; Sigvald, 1984; Thomas *et al*., 1997a). Bagnall and Tai (1886) tested thirty-six potato cultivars in the field for resistance to potato leaf roll virus (PLRV). One hundred and forty eight potato clones/germplasm

were screened at Murree and Faisalabad for the detection of potato leaf roll virus (PLRV), by using enzyme linked immune sorbent assay (ELISA). The potato leaf samples collected from Murree were found to be 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 (Ahmad and Ahmad, 1995). 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, whereas other potato genotypes might resist to infection but susceptible to virus accumulation (Solomon Blackburn 1993; Wilson and Jones, 1993). Therefore the combination of both types of resistance would protect the potato crop from PLRV infection, and its spread could be minimized (Barker and Harrison, 1985; Barker, 1987a).

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	SL15-10	1	R
5	FD63-1	1	R
6	FD78-36	1	R
7	FD76-67	3	MS
8	Sante	1	R
9	FD74-21	1	R
10	FD35-36	2	MR
11	SL5-2	1	R
12	FD76-18	1	R
13	FD61-3	1	R
14	SL9-14	4	S
15	FD73-73	2	MR
16	FD78-51	2	MR
17	SL15-10	1	R
18	SL14-15	1	R
19	SL13-43	2	MR
20	Cardinal	5	HS
LSD =2.024			

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

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	+++	S	2.262
4	SL15-10	_	R	0.325
5	FD63-1	_	R	0.299
6	FD78-36	+	MR	0.978
7	FD76-67	++	MS	1.564
8	Sante	_	R	0.301
9	FD74-21	+	MR	0.879
10	FD35-36	+	MR	0.988
11	SL5-2	+	MR	0.897
12	FD76-18	+	MR	0.889
13	FD61-3	+	MR	0.786
14	SL9-14	+++	S	2.365
15	FD73-73	+	MR	0.876
16	FD78-51	++	MS	1.654
17	SL15-10	+	MR	0.798
18	SL14-15	+	MR	0.998
19	SL13-43	+	MR	0.977
20	cardinal	+++	S	2.367
21	+ve control			2.256
22	+ve control			2.253
23	-ve control			0.976
24	-ve control			0.954

*Deep 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

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