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Evaluation of Potato Lines/Varieties for the Resistant Source against PVX, PVY and PLRV under Natural Field Conditions

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Abstract **– Potato is an important vegetable crop in Pakistan. Reduction in area and production of potato has been observed due to various diseases among them PVX, PVY and PLRV are more important. Yield losses due to these viral diseases are up to 40-80%. Fifty eight varieties/advance lines were screened for the resistant source against PVX, PVY and PLRV in a field trial conducted at research area of Plant Virology Section, Plant Pathology Research Institute, AARI Faisalabad during 2012-2013. Out of 58 lines/varieties on the basis of ELISA results 35 line/varieties including them Hermes, Lady Rosetta and Crusier and other found to free from PVX .Ten lines/varieties showed moderately resistant response and 11 Lines/varieties showed Moderately susceptible response against PVX virus. Two line/varieties showed susceptible response against PVX i.e. 9814 and Desiree. Two varieties/lines were found resistant against PVY i.e. Lady Rosetta, Crusier. 11 were moderately resistant and 7 were moderately susceptible against PVY virus. 34 varieties/lines including them FSD-RED, FSD-WHITE, NARC 394012-962,SH-5, KARODA, Cardinal, Astrix, Sannte, Desiree, FD 69-1 and other were susceptible against PVY. Similarly 19 varieties/lines 19 were found free from PLRV disease. 10 lines/ varieties i.e. FD 78-36, FD 74-51, FD 78-104, FD 76-67, FD 78-3, FD 61-3, NARC, 394574-72, ZS-1, N-34 and SH-704 were moderately resistant against PLRV. 10 varieties/lines were moderately susceptible against PLRV. 19 varieties/lines i.e. including them FD 69-1, FSD –RED, FSD-WHITE, Karoda, Cardinal, Astrix, Diamont, Desiree and other were found susceptible against PLRV disease.**

Keywords **– Potato, PLRV, Resistant, ELISA, Field Conditions**.

I. Introduction

Potato (*Solanum tuberosum* L.) belongs to family Solanaceae the most important vegetable crop being cultivated all over the world. It contains about 79% water, 18 % starch, 2% protein, 1 % minerals and many trace elements. Potato has been appreciated by the growers and relished by consumer due to its high productivity and better quality of starch. The potato crop gives more than 15 times yield per hectare and calories production per unit area is higher than wheat, maize, and rice. There are several factors, which result into low potato production. Among these, diseases such as potato scab, black scurf, early and late blight are important. More than 37 viruses naturally infect potato crop among them potato leaf roll virus (PLRV), potato virus Y (PVY), potato virus X (PVX), potato virus S (PVS), Potato virus A (PVA) and potato virus M (PVM) are prevalent in potato growing areas of Pakistan. Potato leaf roll (PLRV), potato virus X (PVX) and potato virus Y (PVY) are important among six major viruses and these are distributed throughout the Pakistan [1], [2], [3], [4]. Demand of high yielding foreign potato varieties significantly increased the yield of potato crop in Pakistan but at the same time resulted new viral problems which have been reported in spring, summer and autumn potato crop of Pakistan and causes up to 83 % losses [5]. Most viruses can effectively be determined by ELISA tests [6]. At present unfortunately none of the available commercial varieties has full resistance against major three viruses i.e. PVX, PVY and PLRV. Screening of potato germplasm against PVX, PVY and PLRV was the main objective of this study because the genetic control is the cheapest, economical and the most ideal way of managing viral diseases in potato.

II. Materials and Methods

During the winter season 2012-2013 the potato germplasm received from the vegetable research institute, Ayub Agricultural Research Institute (AARI), Potato research Institute, Sahiwal, NARC, Islamabad and Private sector were sown in the research area of Plant Virology Section, Plant Pathology Research Institute, AARI Faisalabad. 58 (fifty eight) varieties/lines/clones was planted in 10 meter ridges at 30 cm plant to plant and 60 cm row to row distance. First irrigation was applied immediately after sowing and then after one to two week intervals. Irrigation was stopped 15 days before harvesting. The potato crop was raised by following recommended agronomic practices. However, no pesticide was sprayed to develop maximum aphid population to increase the disease pressure. A highly susceptible line Desiree was sown as a check after every two test entries as

disease spreader. Natural inoculum was selected upon for infection. Insect traps consisting of plastic cards (1x 1.5 feet) impregnated with Vaseline on both sides, fastened with a hanger were placed in the field at four sides. Data of vector population were taken by counting the number of aphids on yellow boards and on the leaves of the plants. Yellow water traps consisting of yellow iron trays (1x 2.5 feet) were also placed at two sites of potato field and number of aphids trapped in these trays was recorded on weekly basis. The disease incidence of PVX, PVY and PLRV were determined by visual inspection at very line. The incidence of PVX, PVY and PLRV was recorded according to following formula:

$$\text{Disease incidence} = \frac{\text{No of infected plants}}{\text{Total no. of plants}} \times 100$$

Disease rating were taken on weekly basis form October to February during the season. The disease severity was also recorded to find the level of resistance or susceptibility of potato varieties/line on the basis of the following rating scale by [7] and [8].

Confirmation of PLRV through Serological Test (ELISA)

Leaf samples were collected from each variety/line in the month of February 2012-2013 in a plastic envelope and analyzed in ELISA laboratory at Plant Virology section, AARI. All the varieties were analyzed through ELISA. From an individual plant, with or without symptoms leaves were harvested at the bottom, middle and top of the plant and were pooled, thus a composite sample was prepared. The samples were tested for PVX, PVY and PLRV by the double antibody sandwich ELISA (DAS-ELISA) described by Clark and Adams (1977). Each of the leaf samples was homogenized and these were grinded in ELISA buffer. The ELISA plates were coated with 1ml of IgG diluted in 1000 ml coating buffer (prepared by adding 1.59 g of Sodium carbonate (Na_2CO_3) and 2.93 g of Sodium bicarbonate (Na_2HCO_3) in 900 ml distilled water with 9.6 pH to make up the volume up to one liter). After five hours of incubation at 30 °C the samples (200 µl/well) were added in plates and incubated at 6 °C for 16 hours. The plates were washed thrice after each step with washing buffer. After the addition of conjugated IgG (200 µl/well) the plates were incubated at 30 °C for 5 hours and then rinsed thrice with washing buffer. The substrate p-nitrophenyl phosphate was added @ 200 µl/well. Incubation was done at room temperature (25°C) for 30 minutes and reaction visually observed for the development of yellow colour and also read in ELISA reader (model Biotek-L-800) at 405nm for data recording. The reaction was stopped by adding 50µl of 3M NAOH to each well of the plate. The samples showing positive and negative reaction were recorded visually and through ELISA reader reading at 405 nm. The monoclonal ELISA Kits of PVX, PVY and PLRV used during this experiment were obtained from Plant Virology Section, AARI. Based on colour intensity four categories were visualized:

Deep yellow	Strong (+++)	Susceptible
Moderate yellow	Moderate (++)	Moderately susceptible
Mild yellow	Mild (+)	Moderately Resistant
No colour	Free	Resistant

III. Results and Discussion

Out of 58 lines/varieties on the basis of ELISA results 35 line/varieties i.e. FD 35-36, FD 8-11,FD 78-36, FD 74-51, FD 76-18, FD 73-75, FD 78-51, FD 74-8, FD 75-21, FD 74-40, FD 76-12, FD 74-19, FD 78-104, FD 76-67, FD 78-3, FSD-WHITE, FD 69-2, 3912202-158, 393574-61, 394005-115, 394055-40, 396240-21, 396266-33, 394021-120, 396240-181, ZS-1, N-34, SH-704, SH-5, Cardinal, Astrix, Sannte, Hermes, Lady Rosetta and Crusier found free from PVX and thus show resistance against PVX on the basis of ELISA results. 10 lines/varieties i.e. FD 8-1, FD 70-1, FD 75-36, FD 69-1, FD 42-28, FD 48-41, FD 49-28, 394028-37, 394032-16 and NARC 394574-72 showed moderately resistant response against PVX. 11 Lines/varieties showed moderately susceptible response against PVX virus i.e. FD 74-33, FD 70-2, FD 51-5, FD 61-3, FSD-RED, FD 7-2, FD 1-9, NARC 394012-962, 9808, Karoda and Diamont. 2 line/varieties showed susceptible response against PVX i.e. 9814 and Desiree (Table 1). 2 varieties/lines were found resistant against PVY i.e. Lady Rosetta, Crusier. 11 were moderately resistant and 7 were moderately susceptible against PVY virus. 34 varieties/lines i.e. FD 74-104, FSD-RED, FSD-WHITE, FD 7-2, FD 1-9, FD 48-41, FD 49-28, 3912202-158, 393574-61, 394005-115, 394028-37, 394032-16, 394055-40, 396240-21, 396266-33, 394021-120, 396240-181, NARC 394012-962, 9808, 9814, SH-5, KARODA, Cardinal, Astrix, Sannte, Desiree, FD 8-1, FD 74-333, FD 70-1, FD 70-2, FD 75-36, FD 51-5, FD 8-1 and FD 69-1 were susceptible against PVY (Table 1).

Similarly 19 varieties/lines were found free from PLRV disease i.e. FD 8-1, FD 74-333, FD 70-1, FD 75-76, FD 51-5, FD 8-11, FD 76-18, FD 73-75, FD 78-51, FD 74-8, FD 75-21, FD 74-19, FD 69-2, 394021-120, NARC 394012-962,SH-5, Hermes, Lady Rosetta and Crusier. 10 lines/ varieties i.e. FD 78-36, FD 74-51, FD 78-104, FD 76-67, FD 78-3, FD 61-3, NARC, 394574-72, ZS-1, N-34 and SH-704 were moderately resistant against PLRV. 10 varieties/lines were moderately susceptible against PLRV. 19 varieties/lines i.e FD 70-2, FD 35-36, FD 69-1, FSD – RED, FSD-WHITE, FD 1-9, FD 48-41, FD 49-28, 3912202-158, 393574-61, 394032-16, 394055-40, 396240-21, 9808, Karoda, Cardinal, Astrix, Diamont and DESIREE were found susceptible against PLRV disease (Table 1).

Serological study has been conducted by many research workers such as [5] performs ELISA and detected 8 potato viruses to be prevalent in Pakistan. These viruses were; PVX, PVX, PLR, PVS, PVA, PMST and AMV. [9] surveyed and found that PVX, PVY and PLRV were major viral diseases in autumn season in Punjab. In total 169 fields and 1227 samples were analyzed through

ELISA. PVX, PVY and PLRV were present in all localities surveyed. [10] used DAS- ELISA for the detection of PVY, PVX and PLRV. [11] reported six potato viruses; PVX, PVY, PVA, PVS, PVM and PLRV in Nyand ME during 2002-2003 growing season.

Leaf samples were collected in research and commercial potato plots and tested by ELISA. In 2002, 205 symptomatic samples were analyzed; 12 tested for PVA, 36 for PVM, 173 for PVS, 55 for PVX, 182 for PVY and 1 for PLRV with 83% being mixed infection. Several germplasm and varieties/lines are changing with time; new lines are being produced and received from different sources and need to be tested for resistance. Therefore it is a comparative study of old and new varieties. So best known PVX, PVY and PLRV resistance breeding lines and varieties should continue to be collected and put into the breeding programme along with their comparative trials in different suitable geograpgical zones of the country. A strong research programme on the population density, spatial and temporal distribution of *M. persicae* in relation to PVX, PVY and PLRV needs to be initiated.

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Table 1: Response of potato varieties/lines to PVX, PVY and PLRV during 22012-2013

S.#	*Lines/Varieties*	*ELISA REACTION*			*OD VALUE AT 405*			*Level of resistance/susceptibility*		
		PVX	*PVY*	*PLRV*	*PVX*	*PVY*	*PVX*	*PVX*	*PVY*	*PLRV*
1	*FD 8-1*	+	+++	-	*0.91*	*3.281*	*0.416*	*MR*	*S*	*R*
2	*FD 74-333*	++	+++	-	*1.971*	*4.621*	*3.183*	*MS*	*S*	*R*
3	*FD- 70-1*	+	+++	-	*1.213*	*4.546*	*2.062*	*MR*	*S*	*R*
4	*FD 70-2*	++	+++	+++	*1.721*	*3.957*	*2.162*	*MS*	*S*	*S*
5	*FD 35-36*	-	++	-	*0.109*	*1.628*	*0.628*	*R*	*MS*	*S*
6	*FD 75-36*	+	+++	-	*1.356*	*3.466*	*0.621*	*MR*	*S*	*R*
7	*FD 51-5*	++	+++	-	*1.221*	*3.212*	*0.319*	*MS*	*S*	*R*
8	*FD 8-11*	-	+++	+++	*0.06*	*4.414*	*2.560*	*R*	*S*	*R*
9	*FD 69-1*	+	+++	+++	*1.402*	*3.721*	*2.358*	*MR*	*S*	*S*
10	*FD 78-36*	-	+	+	*0.597*	*1.431*	*1.861*	*R*	*MR*	*MR*
11	*FD 74-51*	-	+	+	*0.961*	*1.921*	*1.214*	*R*	*MR*	*MR*
12	*FD 76-18*	-	+	-	*0.782*	*1.721*	*1.210*	*R*	*MR*	*R*
13	*FD 73-75*	-	+	-	*0.458*	*1.891*	*0.777*	*R*	*MR*	*R*
14	*FD 78-51*	-	+	-	*0.566*	*1.653*	*0.541*	*R*	*MR*	*R*
15	*FD 74-8*	-	+	-	*0.691*	*1.482*	*0.498*	*R*	*MR*	*R*
16	*FD 75-21*	-	+	-	*0.062*	*1.222*	*0.201*	*R*	*MR*	*R*
17	*FD 74-40*	-	++	++	*0.101*	*2.268*	*2.561*	*R*	*MS*	*MS*
18	*FD 76-12*	-	++	++	*0.597*	*2.261*	*2.521*	*R*	*MS*	*MS*
19	*FD 74-19*	-	++	-	*0.671*	*2.833*	*0.891*	*R*	*MS*	*R*
20	*FD 78-104*	-	+++	+	*0.861*	*3.686*	*1.913*	*R*	*S*	*MR*
21	*FD 76-67*	-	++	+	*0.361*	*2.622*	*1.353*	*R*	*MS*	*MR*

22	FD 78-3	-	++	+	0.561	2.291	1.291	R	MS	MR
23	FD 61-3	++	+	+	2.618	1.289	1.391	MS	MR	MR
24	FSD- RED	++	+++	+++	2.211	3.576	2.561	MS	S	S
25	FSD- WHITE	-	+++	+++	0.098	3.658	3.416	R	S	S
26	FD 69-2	-	+	-	0.361	1.611	0.353	R	MR	R
27	FD 42-28	+	+++	++	1.391	3.618	1.289	MR	MR	MS
28	FD 7-2	++	+++	++	1.454	3.21	1.371	MS	S	MS
29	FD 1-9	++	+++	+++	1.211	4.191	3.588	MS	S	S
30	FD 48-41	+	+++	+++	0.109	3.614	2.948	MR	S	S
31	FD 49-28	+	+++	+++	1.744	3.876	3.810	MR	S	S
32	3912202-158	-	+++	+++	0.151	4.07	3.271	R	S	S
33	393574-61	-	+++	+++	0.221	3.984	3.144	R	S	S
34	394005-115	-	+++	++	0.196	4.98	2.854	R	S	MS
35	394028-37	+	+++	++	1.062	4.214	2.001	MR	S	MS
36	394032-16	+	+++	+++	1.671	3.861	3.832	MR	S	S
37	394055-40	-	+++	+++	0.153	2.573	3.891	R	S	S
38	396240-21	-	+++	+++	0.24	4.87	4.08	R	S	S
39	396266-33	-	+++	++	0.228	3.629	3.861	R	S	MS
40	394021-120	-	+++	-	0.138	3.621	0.416	R	S	R
41	396240-181	-	+++	++	0.161	3.491	1.741	R	S	MS
42	NARC 394012-962	++	+++	-	2.457	3.631	0.999	MS	S	R
43	NARC 394574-72	+	++	+	0.589	1.721	1.866	MR	MS	MR
44	9808	++	+++	+++	2.961	3.921	4.214	MS	S	S
45	9814	+++	+++	++	2.152	3.782	4.312	S	S	MS
46	ZS-1	-	+	+	0.301	1.268	1.561	R	MR	MR
47	N-34	-	+	+	0.261	1.521	1.671	R	MR	MR
48	SH- 704	-	+	+	0.321	1.721	1.294	R	MR	MR
49	SH-5	-	+++	-	0.101	2.626	0.626	R	S	R
50	KARODA	++	+++	+++	2.417	3.599	4.528	MS	S	S
51	CARDINAL	-	+++	+++	0.08	3.832	3.723	R	S	S
52	ASTRIX	-	+++	++	0.09	3.831	1.683	R	S	S
53	SANNTE	-	+++	++	0.125	3.355	1.355	R	S	MS
54	HERMES	-	++	-	0.652	2.913	0.262	R	MS	R
55	DIAMONT	++	+++	+++	2.561	3.91	3.755	MS	S	S
56	LADY ROSETA	-	-	-	0.09	0.691	0.791	R	R	R
57	CRUSIER	-	-	-	0.13	0.56	0.76	R	R	R
58	DESIREE (Check)	+++	+++	+++	2.223	4.314	3.191	S	S	S