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Resistance status of potato germplasm against leaf roll virus under natural field conditions

Azher Mustafa[1], Muhammad Mohsan[1], Muhammad Rizwan Bashir[2*], Muhammad Iqbal[1], Saba Saeed[1], Sajid Hussain Zaidi[1], Arif Muhammad Khan[3], Muhammad Kashif Hanif[3]

[1]*Plant Virology Section, Plant Pathology Research Institute Faisalabad, Pakistan*

[2]*Oilseeds Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan*

[3]*Department of Biotechnology University of Sargodha, Pakistan*

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Abstract

Potato is the most important crop packed with nutritional benefits needed for a healthy lifestyle. Among various devastating diseases potato leaf roll virus (PLRV) causes significant decrease in quality and quantity of potato crop. In the current research potato germplasm received from Potato Research Institute (PRI), Sahiwal, Pakistan was sown at Plant Virology Section, Plant Pathology Research Institute, Faisalabad to study the varietal reaction against PLRV. No variety/ advanced line exhibited highly resistant response. Only four varieties/ lines *viz*.FD 74-38, FD 73-38, FD 78-36 and Rubi showed moderately resistant reaction whereas nine varieties/lines namely FD 76-78, FD 73-110, SL 11-50, FD 76-35, FD 76-67, FD 74-28, FD 76-30, PRI RED and FD 76-72expressed moderately susceptible response. Likewise, twelve potato varieties/ advanced lines such as FD 77-4, FSD WHITE, FSD RED, FD 69-1, FD 76-59, FD 71-1, FD 36-36, SL 10-4, FD 78-51, FD 8-1, FD 74-30 and SL 8-5 proved to be susceptible while four varieties/lines i.e.SL 9-13, FD 74-50, FD 78-15 and FD 76-55exhibitedhighly susceptible response by using disease rating scale. It is concluded that potato varieties/ advanced lines that expressed moderately resistant response might be used for general cultivation by managing all other yield limiting factors such as nutritional deficiency, irrigation, fertilizers, insects and tillage operations etc.

* **Corresponding Author:** Muhammad Rizwan Bashir ✉ mrizwan1526@gmail.com

Introduction

Potato (*Solanum tuberosum* L.) is an important vegetable crop and it fall at fourth number after rice, maize and wheat (Hopp *et al.*, 1988). We can achieve more yield and calories from per unit area than other crop (McGillivray, 1953).

It has a potential to serve as a valuable food for the ever increasing world population as it contains higher dry matter, higher protein production and it is adaptable to wide range of climatic conditions. It also provides protein, minerals, carbohydrates, vitamins (B & C) and high quality dietary fibres (Swaminathan, 1962). More than thirty five plant viruses infect potato crop (Brunt *et al.*, 1989). A lower incidence (5-10%) of the viruses (singly or in combination) infections in the current or previous crop slightly reduces the yields of crop. A higher virus incidence coupled with early or severe infection pose a serious threat to tuber yield (Garg, 1987). Accurate estimates about the economic losses caused by viruses are lacking in potato crop. It was estimated roughly that potato viruses may cause yield losses up to 50% in tuber yield. In Pakistan, climatic and soil conditions are highly favourable for autumn, spring and summer potato crops (Zanoni, 1991).

Among viral diseases, PVX, PVY, PLRV, PVA, PVM, PVS and PMTV have been observed and caused up to 83% yield losses in potato crop (Mughal and Khalid, 1985). PVX, PVS, PVA, PVM and PVY are more common viruses while PLRV is prevalent in northern areas where insect vector pressure is high. PLRV is devastating disease that affects both yield as well as quality of the produce. Aphid is carrier of PLRV in spring planted crop (Mirza, 1978). Two types of symptoms are seen in field as a result of PLRV attack i.e. primary and secondary symptoms. In case of primary symptoms, upper leaves are affected, curling of leaves and reduction of leaf size of upper leaves. Primary symptoms are produce as a result of aphid attack. Secondary symptoms are produce when infected rubbers are sown in field. Leaf curling, leaf thickness and red pigmentation of leaf margins are secondary symptoms.

Less resistance and high severity of this disease in most of varieties/lines is because of high inoculums level which may continue to introduce (Umar *et al.,* 2011). There is dire need to promote more resistant cultivar of potato because of complexity of other methods. Now a day it is important to introduce substituted advanced crop protection or control methods. With the less use of conventional synthetic chemicals and bio pesticide, pathologist and breeders have to develop resistant cultivar of potato because it is also a cost limiting factor. In this way the screening of resistance source is encouraged in all important crops.

Materials and methods

Site selection and germplasm collection

The trial was sown at Plant Virology Section Research Area on 21-11-2016. Twenty nine lines/cultivars were sown under Randomised Complete Block Design (RCBD) with three replications. These lines were received from Potato Research Institute, Sahiwal.

Sowing of potato germplasm

Twenty five tubers of each variety /line were sown on ridges with plant to plant distance of 30cm. The ridges were 60 cm apart. One row of susceptible check variety (Desiree) was planted after every tenth variety. Three bags of DAP and one bag of Potash @ per acre were added to soil after preparing the field. The field was irrigated after eight days interval. Four bags of urea @ per acre were applied at different growth stages of crop. Metribuzin was spayed for the control of weeds. No insectide was sprayed to invite the maximum population of aphid.

Data collection

Data of disease were recorded when susceptible check showed maximum appearance of leaf curling according to the disease rating scale given by(Khan, 2002). Five plants from each row were selected and disease was assessed by disease rating scale.

Confirmation of disease through ELISA test

The virus was detected by Enzyme-linked immunosorbent assay (ELISA) method (Clark and

Adam, 1997). Bioreba kit was used for this method. 96 wells ELISA plate was coated with coating antibodies after adding coating antibody with coating buffer. Coating buffer was prepared by adding on tablet of blue colour in 100 ml distil water. Each well received 100 ul coating antibodies. The coated ELISA plate was incubated at 4°C for one day. Next day ELISA plate was washed with washing buffer after removing the coating buffer from wells. This practice was performed in sink and repeated thrice. Symptomatic leaves were collected from field and put in extraction bags. Extraction buffer wad added and leaves were crushed to obtain antigen (virus). 100 ulantigens were added to each well. First two wells received positive control and last two were loaded with negative control. Plates were incubated at 4°C for 24 hours. Next day washing was carried out with washing buffer according to procedure as described earlier. Conjugate antibody mixed with conjugate buffer was added to each well @100 *ul.* Elisa plates were incubated at 4°C for one day. Washing was carried out as described earlier. Substrate buffer mixed with pnptablet (1mg/ml) was poured at the end and reaction was stoped with 3Moler NaOH. Yellow colour was assessed visually and reading was recorded by ELISA reader using software Gen Five at 405 nm.

Statistical analysis

All the statistical tests were performed by using SAS statistical software (SAS Institute, 1990). Means were separated by using Fisher's protected least significant difference (LSD) procedure by taking P = 0.05% probability level (Steel, *et al.,* 1997). Analysis of variance (ANOVA), interaction of different treatments and their combinations were developed by using SAS/STAT software package.

Results

All the cultivars came out symptomatic under field condition. This showed the presence of virus in all cultivars. FD 74-38, FD 73-38, FD 78-36 and Ruby showed moderately resistant (MR) response to PLRV. Nine cultivars (FD 76-78, FD 73-110, SL 11-50, FD 76-35, FD 76-67, FD 74-28, FD 76-30, PRI RED and FD 76-72) graded as moderately susceptible to PLRV. Twelve varieties/lines (FD 77-4, FSD WHITE, FSD RED, FD 69-1, FD 76-59, FD 71-1, FD 36-36, SL 10-4, FD 78-51, FD 8-1, FD 74-30 and SL 8-5) proved to be susceptible. Four varieties/lines (SL 9-13, FD 74-50, FD 78-15 and FD 76-55) came out as highly susceptible. All the samples gave yellow colour which showed the presence of antigen i.e. FD 74-38, FD 73-38, FD 78-36 rubi, FD 76-78, FD 73-110, SL 11-50, FD 76-35, FD 76-67, FD 74-28, FD 76-30, PRI RED, FD 76-72, FD 77-4, FSD WHITE, FSD RED, FD 69-1, FD 76-59, FD 71-1, FD 36-36, SL 10-4, FD 78-51, FD 8-1, FD 74-30, SL 8-5, SL 9-13, FD 74-50, FD 78-15 and FD 76-bright yellow colour indicate the strong frequency of virus and light reflect the low virus frequency.

Table 1. Disease rating scale of Khan *et al.,* (2002) against potato leaf roll virus PLRV.

Sr. No.	Description	Response
0	No symptoms	HR
1	Rolling of upper leaves (Primary infection)	R
2	Rolling of upper and lower leaves (Secondary infection), erect growth	MR
3	Rolling of leaves extending, leaves become stiff and leathery, stunting of plants and erect growth.	MS
4	Short internodes, papery sound of leathery leaves, rolling and stunting of whole plants. Young buds are slight yellowish and purplish.	S
5	Clear rolling of leaves, sever stunting, few tubers and tuber necrosis	HS

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

Discussion

In current study few varieties i.e. FD 74-38, FD 73-38, FD 78-36 and Rubi proved moderately resistant, some varieties were found susceptible while FD 76-78, FD 73-110, SL 11-50, FD 76-35, FD 76-67, FD 74-28, FD 76-30, PRI RED and FD 76-72) graded as moderately susceptible. Our results are in line with the findings of khan (2002) who tested 15 varieties/lines against potato leaf roll virus under natural field condition but none of the tested variety/line behaved as resistant. Same type of research was also performed by Ahmad and Aman(2003) but they also could not find out resistant variety/line against PLRV. Diseased samples were subjected to ELISA test for virus confirmation. Diseased symptoms are not 100 surety of virus prescence in plant samples as many other factors like rhizoctonia attack, potato virus M and environmental factors produce same type of symptos. Therefore virus is detected by ELISA technique which is a protein based method.

Table 2. Response of different varieties/ lines against PLRV.

Sr. No.	Variety/ Line	DiseaseSeverity Index	Levelof Resistance	ELISA Reaction OD at 405nm
1	FD 76-78	3	MS	2.02
2	FD 77-4	4	S	3.25
3	FD 73-110	3	MS	1.79
4	SL 11-50	3	MS	2.08
5	FSD WHITE	4	S	3.33
6	FD 74-38	2	MR	1.02
7	FSD RED	4	S	3.56
8	FD 69-1	4	S	3.44
9	FD 76-59	4	S	3.76
10	FD 73-38	2	MR	.98
11	FD 71-1	4	S	3.56
12	FD 78-36	2	MR	1.06
13	FD 76-35	3	MS	2.67
14	FD 35-36	4	S	3.66
15	SL 10-4	4	S	2.99
16	FD 78-51	4	S	3.77
17	FD 76-67	3	MS	2.84
18	FD 8-1	4	S	3.54
19	FD 74-30	4	S	3.78
20	SL 9-13	5	HS	4.06
21	FD 74-28	3	MS	2.51
22	FD 76-30	3	MS	2.55
23	PRI RED	3	MS	2.77
24	FD 76-72	3	MS	2.87
25	SL 8-5	4	S	3.89
26	FD 74-50	5	HS	4.06
27	RUBI	2	MR	1.00
28	FD 78-15	5	HS	4.88
29	FD 76-55	5	HS	4.00

This is a reliable method and used by many labs but one limitation of this method is that thid procedure detect virus on protein basis not on nucleic acid basis. But owing to dearth of facility at our lab we opted for ELISA method. Mughal*et al.*, (1998) detected eight potato viruses from Pakistan by utilizing ELISA techniques. Indicators plants are also another option but it is an old method for virus detection. Similarly, Ahmad *et al.*,(2003) conducted a research trial by recording survey of PLRV in seven districts of Punjab. They collected 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.

Conclusion

In the current research only four varieties/lines *viz.* FD 74-38, FD 73-38, FD 78-36 and Rubi expressed moderately resistant response against the PLRV. Nine varieties/lines *i.e.* FD 76-78, FD 73-110, SL 11-50, FD 76-35, FD 76-67, FD 74-28, FD 76-30, PRI RED and FD 76-72showed moderately susceptible response. Likewise, twelve varieties/advanced lines such as FD 77-4, FSD WHITE, FSD RED, FD 69-1, FD 76-59, FD 71-1, FD 36-36, SL 10-4, FD 78-51, FD 8-1, FD 74-30 and SL 8-5 exhibited susceptible response whereas four varieties/lines namely SL 9-13, FD 74-50, FD 78-15 AND FD 76-55 expressed highly susceptible response.

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