INT. J. BIOL. BIOTECH., 10 (4): 645-650, 2013.

ELISA BASED SCREENING OF POTATO VARIETIES / LINES AGAINST POTATO VIRUSES

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ABSTRACT

The reaction of 20 potato genotypes was determined using double antibody sandwich ELISA (DAS- ELISA) against six viruses viz; Potato Leaf Roll Virus (PLRV), Potato Virus X (PVX), Potato Virus Y (PVY), Potato Virus S (PVS), Potato Virus A (PVA) and Potato Virus M (PVM). The study was conducted at Potato Research Institute, Sahiwal during 2010-11 and 2011-12. The samples were collected from the field and tested in the laboratory of Plant Pathology at Potato Research Institute, Sahiwal. The reaction of ELISA for different viruses showed that out of 20 genotypes, different responses were detected with varying percentage against six potato viruses, some shows moderately susceptible behavior against potato viruses and some shows susceptible behavior: susceptible response means that there is not a single gene present which can resist against potato viruses. Five genotypes viz; FD 75-47, SH-5, FD 76-12, FD 74-33 and FD 74-8 showed the resistance response against all the potato viruses in both years experiments. This indicates that these 5 genotypes are the good source of resistance and may be useful for the development of potato cultivars resistant to potato viruses.

Key-words: Screening; Potato; Viruses; Varieties/Lines, ELISA

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most widely distributed crop in tropical and subtropical zones of the world. In Pakistan, potato is cultivated over an area of 185.0 thousand hectares with production of 4104.4 thousand tons (Anon., 2012). The potato is produced in three seasons i.e. spring, summer and autumn. Its average yield is 20.5 tons per hectare which is very low as compared to other countries of the world. There are many factors which hinder its yield. Among these non-availability of good quality seed potato and potato diseases are important. Among diseases the potato viruses play major role in reducing the yield (Chaudhary *et al.*, 1990) because these are difficult to manage due to non-availability of chemicals for direct control. Mughal and Khalid (1985) have reported potato crop losses upto 83 percent due to viruses in Pakistan. Ahmad and Bhutta (1989) found PVY, PVX and PLRV as major viral diseases occurring in Pakistan. Ahmad *et al.* (2003) found 13.18 and 23.06 percent samples infected with PVX and PVY, respectively in seven main potato growing districts of Punjab. Schneller *et al.* (2003) collected 205 leaf samples and tested by ELISA; 55 samples were positive for PVX, 173 for PVS and 182 for PVY. Disease incidence of PVY in potato ranges between 5-25% in Pakistan (Mughal *et al*., 1988) and it can destroy the whole crop if it occurs along with PVX and PVS. Gray *et al.* (2003) reported a high percentage of symptomatic plants infected with PVY and PVS. Ahmad and Ahmad (1995) found PVX and PVY as major viruses in the autumn season in the Punjab. Most of the potato viruses were perpetuated through seed tubers. The concentration and percentage of diseased tubers were increased by infected seed year after year Jafarpour *et al.* (1986). Currently, none of the available high yielding commercial varieties/advance lines has shown durable resistance against these diseases (Qamar *et al*., 2003). This is mainly due to the presence of disease virulence of these viruses (Ahmad & Ahmad, 1995), continuous introductions of the viruses through imported seeds, recurrent occurrence of the carrier/vector of these diseases i.e. aphid (*Myzus persicae* Schulz), non availability of chemical substances for directly controlling viral diseases of plants in the field.

MATERIALS AND METHODS

The procedure for double antibody sandwich ELISA (DAS- ELISA) involved the following steps;

Step 1

- Dissolved 20µl antibody of six viruses in 20 ml antibody coating buffer separately for each virus and made the desired solution.
- Coated the solution @ 200µl for each well in the microtitre plate with the help of micropipette.
- Then incubated the microtitre plate at 4^{o} C overnight

Step 2

Leaf samples of different genotypes were collected for testing through ELISA against Potato Leaf Roll Virus (PLRV), Potato Virus X (PVX), Potato Virus Y (PVY), Potato Virus S (PVS), Potato Virus A (PVA) and Potato Virus M (PVM) as described by Clark and Adam (1977).

- After incubation the plates were washed with washing buffer 3 times.
- The collected leaf samples mixed with virus extraction buffer @ 1:10 with the help of mortar and pestle to extract the sap and homogenized. The six different plates for six viruses were filled with the sap @ 200µl for each well with the help of micropipette.
- Before adding the samples into the plates, filled 2 wells for the positive control and 2 for the negative control of the six viruses separately.
- The coated plate was again incubated for overnight at 4^{o} C.

Step 3

- Washed the plates with washing buffer 3times and then take conjugate 20µl and added conjugate buffer 20ml separately for each virus.
- Poured the plates at the rate of 200µl for each well.
- Incubated the plate at 4^{o} C overnight.

Step 4

- Washed the plate 3 times with washing buffer and then take 20ml substrate buffer and 1 PNP tablet.
- Mixed tablet in substrate buffer.
- Poured the plates @ 200µl for each well with the help of micropipette.
- Put the plate for 30 minutes at room temperature and reaction was visually observed for the development of yellow color.
- The reaction was stopped by adding 50µl 3M NaOH to each well.
- The results were compiled by the following scale

Scale for ELISA Results;

➢ Deep Yellow =	strong (+++) =	Susceptible
➢ Moderate Yellow =	Moderate(++) =	Moderately Susceptible
➢ No color =	Free =	Resistant

RESULTS AND DISCUSSION

Development of yellow color in the wells indicated the presence of a virus and its intensity which is proportional to the concentration of virus in the plant. Therefore, the positive and negative samples were stored out by visual observation of yellow color development of enzyme.

Positive reaction was observed with some PLRV infected leaves. The color reaction was moderate yellow to dark yellow. All the varieties and lines were subjected to double antibody sandwich ELISA (DAS- ELISA), using monoclonal antibodies (Clark and Adam, 1977). The genotype FD 49-62 exhibited susceptible response to PLRV in two years study during 2010-11 and 2011-12; five genotypes viz: FD 69-2, FD-48-4, cardinal, FD 73-13 and SH-704 showed moderately susceptible response to PLRV but SH-704 showed resistance in 2011-2012; all other genotypes were resistance to PLRV in both years experiments (Table 1 & 2). These findings are in conformity with earlier work (Batool *et al.*, 2011), where 29 potato varieties/lines were screened against potato leaf roll virus (PLRV). The results showed that only 25 varieties /lines were ELISA positive and 4 genotypes showed negative results against PLRV.

For PVX three genotypes viz: FD 48-4, Cardinal, FD 37-13 and FD 49-62 were exhibited susceptible in both year study; while six genotypes FD69-1, FD63-1, FD-35-36 and FD-1-3, FD69-2 and Cardinal were exhibited moderately susceptible while Cardinal was susceptible and FD 69-2 was resistance to PVX in the first year study; eleven genotypes FD 75-47, FD 70-1, FD 61-3, FD 69-2, SH-704, SH-692, SH-5, FD 76-12, N-2002-1, FD 74-8 and FD 8-1 were shown resistance in the first year study while FD 69-1, FD 37-13, FD 1-3 were also show resistant to PVX in the second year study. Schneller *et al.* (2003) reported six potato viruses; PVX, PVY, PVA, PVS, PVM and PLRV in Ny and 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. Baldauf *et al.* (2006) conducted a survey of six potato viruses, Potato virus A (PVA), Potato virus M (PVM), Potato virus S (PVS), Potato virus X (PVX), Potato virus Y (PVY), and Potato leaf roll virus (PLRV), in New York and Maine during 2002 and 2003. Leaf samples were tested by enzyme linked immunosorbent assay (ELISA).

For PVY four genotypes FD 63-1, FD 48-4, Cardinal and SH-788 exhibited susceptible response but FD 63-1 showed moderately susceptible response in the second year experiment; four genotypes FD 35-36, FD 49-62, FD 37-13 and FD 1-3 were moderately susceptible while only one genotype FD 49-62 was moderately susceptible in the second year experiment and thirteen genotypes viz.: FD 69-1, FD 75-47, FD 70-1, FD 61-3, FD 69-2, SH-704, SH-692, SH-5, FD 76-12 , N-2002-1, FD 74-33, FD 74-8 and FD 8-1 were exhibited resistance response although FD 35-36, FD 37-13 and FD 1-3 were also showed resistance behavior against PVY in the second year experiment in 2011-2012. Jarjees (2000) studied the application of enzyme linked immunosorbent assay for rapid detection of PVY in Iraq. The virus was isolated from naturally infected potato plants and identified as symptoms produced on diagnostic host and through serological test. The virus was purified and used for antiserum production. Results revealed that ELISA could detect rapidly even at low concentration in purified preparation as well as in sap of infected plants. Abou-Jawdah *et al.* (2001) studied potato fields in the two main production areas of Lebanon, the Bekaa and Akkar plains, for viruses and other pathogens of significance for a potato seed certification programme. ELISA tests showed that PVY was the predominant virus, followed by PVS and PLRV. Of 789 samples tested by ELISA, 372 samples were infected by one or more viruses. Single, double and multiple infections represented 75.3, 21.2 and 3.5% of all infected samples, respectively. Incidence of viruses was higher on crops from locally produced uncertified seed potatoes than on crops from imported certified seed potatoes.

Two genotypes Cardinal and FD 49-62 exhibited susceptible response to PVS in both the years except Cardinal which shows moderately susceptible response in the 2011-2012; seven genotypes viz.: FD 69-1, FD 69-2, N-2002-1, FD 48-4, FD 35-36, FD 1-3 and FD 37-13 showed moderately susceptible response to PVS in the first year experiment during 2010-2011 while eight genotypes FD 63-1, FD 69-2, N-2002-1, FD 48-4, Cardinal FD 35-36, FD 1-3 and FD 37-13 showed Moderately susceptible response to PVS under DAS-ELISA and eleven genotypes viz : FD 75-43, FD 70-1, FD 61-3, FD 63-1, SH-704, SH-692, SH-5, FD 76-12, FD 74-33, FD 74-8 and FD 8-1 were resistance to PVS in the first year experiment; eleven genotypes also showed resistant response in the second year experiment in 2011-12. Results given in Table-1 and Table-2 clearly indicate that there was positive reaction with PVS used as antigen agent with PVS monoclonal antibodies, whereas healthy tissues gave negative reaction and these results clearly revealed that PVS was not present in the field samples of potato genotypes but PVS was present in some of the potato genotypes.

Similarly positive reaction was also observed with PVA infected tissues. The color reaction was moderate yellow to dark yellow. All the varieties and lines were subjected to double antibody sandwich ELISA (DAS-ELISA), using monoclonal antibodies (Clark and Adam, 1977). It provided rapid, reliable and accurate diagnosis of PVA. The results in the tables (1 & 2) showed that four genotypes viz.: SH-692, N-2002-1, Cardinal and FD 49-62 were exhibited susceptible to PVA in the first year experiment while only one variety was susceptible in the second year experiment in 2011-12 which was FD 49-62; eleven genotypes viz.: FD 69-1, FD 70-1, FD 61-3, FD 63-1, FD 69-2, SH-704, FD 48-4, FD 35-36, FD 37-13, FD 1-3 and FD 8-1 were exhibited moderately susceptible response to PVA in the first year's experiment while twelve genotypes showed moderately susceptible response in the 2nd year's experiment in 2011-12 which were FD 69-1, FD 61-3, FD 63-1, FD 69-2, SH-704 SH-692, N-2002-1, Cardinal, FD 48-4, FD 35-36, FD 37-13, and FD 1-3; five genotypes viz: FD 75-47, SH-5, FD-76-12, FD-74-33 and FD 74-8 were shown resistance to PVA in 2010-11 and seven genotypes exhibited resistant response to PVA in 2011-12. Schneller *et al*. (2003) also reported PVA in Nyand ME during 2002-2003 growing season. Leaf samples of 12 plants were collected in research and commercial potato plots and tested by ELISA. It is estimated that 83 % of the samples showed the reaction.

For PVM Tables (1 & 2) showed that four genotypes SH-692, Cardinal, FD 49-62 and FD 37-13 exhibited susceptible response in 2010-11 while only one genotype FD-49-62 showed susceptible response in 2011-12; four genotypes FD 75-47, SH-5, FD-76-12, and FD-74-33 were resistance to PVM in 2010-11 while seven genotypes FD 75-47, FD 70-1, SH-5, FD 76-12, FD 74-33, FD 74-8 and FD 8-1 exhibited resistance response to PVM; eleven genotypes FD 69-1, FD 61-3, FD 63-1, FD 69-2, SH-704, N-2002-1, FD 48-4, Cardinal, FD 35-36, FD 37-13 and FD 1-3 were moderately susceptible in 2010-11 while twelve genotypes were moderately susceptible in the 2nd year experiment in 2011-12. Schneller *et al.* (2003) also reported six potato viruses; PVX, PVY, PVA, PVS, PVN and PLRV in Ny and 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. Zhang (2004) also studied more than 2000 samples of potato infected by viruses, tissue culture potato and mini potato tubers were deducted and identified by Electron microscopy which was collected from main potato growing areas of Yunnan. PVX, PVY, PLRV, PMTV and PVM were detected and potato yield reduced evidently along the raising virus infection rate. Virus was detected through TAS- ELISA and electron microscopy.

ACKNOWLEDGEMENTS

Author is grateful to Potato Research Institute, Sahiwal, Pakistan for providing the facilities to carry out this research work and is also thankful to Government of Punjab, Pakistan for providing grant for the research work.

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(*Accepted for publication September 2013*)

Table 1. Results of Enzyme linked Immunosorbent Assay (ELISA) of six potato viruses during 2010-2011.

Sr. No	Genotypes	PLRV		PVX		PVY		PVS		PVA		PVM	
		Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility
1	FD 69-1	-	R	++	MS	-	R	++	MS	++	MS	++	MS
2	FD75-47	-	R	-	R	-	R	-	R	-	R	-	R
3	FD 70-1	-	R	-	R	-	R	-	R	++	MS	++	MS
4	FD 61-3	-	R	-	R	-	R	-	R	++	MS	++	MS
5	FD 63-1	-	R	++	MS	+++	S	-	R	++	MS	++	MS
6	FD 69-2	-	R	-	R	-	R	++	MS	++	MS	++	MS
7	SH-704	++	MS	-	R	-	R	-	R	++	MS	++	MS
8	SH-692	-	R	-	R	-	R	-	R	+++	S	+++	S
9	SH-5	-	R	-	R	-	R	-	R	-	R	-	R
10	FD76-12	-	R	-	R	-	R	-	R	-	R	-	R
11	N-2002-1	-	R	-	R	-	R	++	MS	+++	S	++	MS
12	FD 48-4	++	MS	+++	S	+++	S	++	MS	++	MS	++	MS
13	Cardinal	++	MS	+++	S	+++	S	+++	S	+++	S	+++	S
14	FD74-33	-	R	-	R	-	R	-	R	-	R	-	R
15	FD 35-36	-	R	++	MS	++	MS	++	MS	++	MS	++	MS
16	FD 49-62	+++	S	+++	S	++	MS	+++	S	+++	S	+++	S
17	FD74-8	-	R	-	R	-	R	-	R	-	R	-	R
18	FD 37-13	++	MS	+++	S	++	MS	++	MS	++	MS	+++	S
19	FD 1-3	-	R	++	MS	++	MS	++	MS	++	MS	++	MS
20	FD 8-1	-	R	-	R	-	R	-	R	++	MS	++	MS

Table 2. Results of Enzyme linked Immunosorbent Assay (ELISA) of six Potato Viruses during 2011-2012

Sr. No	Genotypes	PLRV		PVX		PVY		PVS		PVA		PVM	
		Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility	Scale for ELISA	Level of Resistant / Susceptibility
1	FD 69-1	-	R	-	R	-	R	-	R	++	MS	++	MS
2	FD75-47	-	R	-	R	-	R	-	R	-	R	-	R
3	FD70-1	-	R	-	R	-	R	-	R	-	R	-	R
4	FD 61-3	-	R	-	R	-	R	-	R	++	MS	++	MS
5	FD 63-1	-	R	++	MS	++	MS	++	MS	++	MS	++	MS
6	FD 69-2	++	MS	++	MS	-	R	++	MS	++	MS	++	MS
7	SH-704	-	R	-	R	-	R	-	R	++	MS	++	MS
8	SH-692	-	R	-	R	-	R	-	R	++	MS	++	MS
9	SH-5	-	R	-	R	-	R	-	R	-	R	-	R
10	FD76-12	-	R	-	R	-	R	-	R	-	R	-	R
11	N-2002-1	-	R	-	R	-	R	++	MS	++	MS	++	MS
12	FD 48-4	++	MS	+++	S	+++	S	++	MS	++	MS	++	MS
13	Cardinal	++	MS	++	MS	+++	S	++	MS	++	MS	++	MS
14	FD74-33	-	R	-	R	-	R	-	R	-	R	-	R
15	FD 35-36	-	R	++	MS	-	R	++	MS	++	MS	++	MS
16	FD 49-62	+++	S	+++	S	++	MS	+++	S	+++	S	+++	S
17	FD74-8	-	R	-	R	-	R	-	R	-	R	-	R
18	FD 37-13	++	MS	-	R	-	R	++	MS	++	MS	++	MS
19	FD 1-3	-	R	-	R	-	R	++	MS	++	MS	++	MS
20	FD 8-1	-	R	-	R	-	R	-	R	-	R	-	R