Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.8, 2014

Study of Important Soil-Borne Viruses in Major Potato Growing Areas of Hazara and Malakand Divisions Khyber Pakhtoonkhaw-Pakistan

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Abstract
To study important soil-borne viruses of potato an extensive field surveys were conducted in Hazara and Malakand Divisions during potato growing seasons in 2011-12. Incidence and distribution of Potato mop-top pomovirus (PMTV) and Tobacco rattle tobra virus (TRV) was reported on the basis of characteristic symptoms expression, bait test, infectivity assays and double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). Both the viruses were prevalent in Hazara and Malakand divisions. The average symptoms based incidence in Hazara division for PMTV was 8.83% and TRV was 6.80% while in Malakand division the average symptoms based incidence for PMTV was 8.46% and TRV was 9.95%. DAS-ELISA based average incidence in Hazara of PMTV and TRV was 17.34% and 11.99% respectively. The average DAS-ELISA based incidence in Malakand division for PMTV was 21.36% and TRV was 13.71%. The highest symptom based incidence of PMTV in the Hazara was in Baffa 10.75% and in Kalam 10.34% was in Malakand division whereas the highest incidence of TRV in Hazara was in Gojri 8.08% and in Malakand was in Shangla-top 11.62 %. The highest incidence of PMTV based on ELISA was recorded in samples collected from the farmers field at Baffa (21.73%) of Hazara while (23.80%) in Kalam of Malakand division. The highest incidence of TRV based on ELISA in Hazara was found in Mangal 14.28% and in Malakand division the highest incidence was in Shangla Top 17.39%. Isolates of PMTV and TRV were characterized using biological and serological techniques. Biological characterization of the field isolates revealed that variability in the symptoms produced by them on host plant. The interaction and association of viruses (PMTV and TRV) and their vectors was calculated by using Jaccard index of similarity. The association of PMTV to its vector *Spongosporsa subteranea* in Hazara division was found 27.0% while their association in Malakand division was found 24.4%. The TRV associated to its vectors Trichodorus in Hazara division was found 8.94% while in Malakand division was 20.7%. It was concluded that molecular studies will be required to determine variability among the prevalent isolates of PMTV and TRV.
Keywords: Virus, Nematode, Fungus, Incidence, Jaccard index of similarity.

INTRODUCTION

The genus "*Solanum*", family "*Solanaceae*", includes the tuber-bearing species, of which the cultivated potato "*Solanum tuberosum* ssp. *tuberosum*" is best known (Huaman and Spooner, 2002). Worldwide, it is the most widely distributed crop in tropical and subtropical zones of the world and potato is one of the most important widely grown crops, ranking fourth after wheat, maize, and rice, respectively (Stevenson *et al.,* 2001). Potato is susceptible to pathogens, such as viruses, viroids, fungi, bacteria, phytoplasmas, and nematodes. Potatoes are naturally susceptible to about forty viruses and two viroids (Valkonen, 2007). Viruses are the most important pathogens of potato, because they are transmitted in symptomless seed-tubers from one generation to other. Among viruses, soil-borne viruses particularly *Potato mop top pomovirus* (PMTV) and *Tobacco rattle tobravirus* (TRV) gained critical importance due to perpetuation in tuber and non-availability of host resistance. PMTV, member of the genus *pomovirus,* particles are tubular rod-shaped and genome consists of tripartite single-stranded, positive sense RNA molecules (McGeachy and Barker, 2000). Initially the transmission of PMTV occurs in potato by the infection of powdery scab pathogen *Spongospora subterranea* f.sp. *subterranea*, a plasmodiophorid vector. Powdery scab is prevalent worldwide (Arif *et al.*, 1995). Another virus that infects potato-tubers is *Tobacco rattle tobravirus* (TRV). TRV is tubular rod-shaped particles with two prominent lengths such as 185-196 nm and 50-115 nm, having RNA 1 and RNA 2, respectively (Robinson, 2003). *Trichodorus* and *Paratrichodorus* belong to the family *Trichoridae*. Nematodes of this family are rounded at the end and 0.35-1.8 mm long (Boutsika *et al*. 2004). Nematodes are also important due to their ability to transmit tobraviruses (Holeva et al., 2006). PMTV was reported in potato growing areas of Hazara and Malakand divisions (M. Arif, personal communication). However, nothing is known about the TRV in major potato growing regions of Khyber Pakhtunkhwa province. Comprehensive study was needed to investigate soil-borne viruses in Hazara and Malakand divisions. This study was conducted with following objectives:

1. To determine the incidence and distribution of major soil borne-viruses (PMTV and TRV) and their vectors in major potato growing areas of Hazara and Malakand divisions.

2. To identify and characterize major soil-borne viruses (PMTV and TRV) in potato.

MATERIALS AND METHODS

A survey was conducted on seven locations from Hazara Division and eight locations of Malakand division. Soil sample was collected along with the plants and tubers showing the characteristic symptoms of PMTV and TRV and were brought to the Plant Pathology Laboratory at The University of Agriculture Peshawar, for further studies. Incidence of both virus were calculated by the following formula

$$\text{Percent incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Number of plants in the field}} \times 100$$

Soil samples collected were processed as described by Whitney, (1989) and Arif *et al.,* (1994) for PMTV and for TRV fresh soil samples were used in bait test (Mojtahedi and Santo, 1999). For Nematodes soil samples were processed for the extraction by sieving method (Boag and Brown, 1988) and Nematodes were collected in centrifuge tube. After centrifugation a suspension of pellet was prepared in order to carry out ELISA to check the presence of Viruliferous Nematodes. The experiments were laid out for PMTV and TRV in CRD having 15 treatments and 3 replications. Potato susceptible variety Rocoo was grown for PMTV and Desiree for TRV. Potato germplasm was grown for about three months in infested soil in bait test whereas bait test plant *N. benthamiana* was grown after 4-6 weeks. Susceptible potato and other test plants were evaluated on the basis of visual symptoms and back indexing of the suspected plants on *Nicotiana debneyi* and *Chenopodium amaranticolor.* (Harrison and Reavy, 2002). Back indexing test based on sap inoculation was conducted to prove the identity of PMTV and TRV in screen house. DAS-ELISA was performed for the confirmation of PMTV/TRV and to calculate the incidence. The analysis was done by using Statistix 8 (analytical software) package.

RESULTS AND DISCUSSION

Symptoms based Incidence of PMTV

On the basis of field symptoms the incidence of PMTV was observed both in Hazara and Malakand Division. The table shows highest incidence in the Baffa area of Hazara was more (10.75%) as compared to other localities of the Division. In Malakand Division the highest incidence of PMTV was calculated in Kalam (10.34%). The minimum incidence observed was 7.22% in Ghandian area of Hazara and Kotkay (7.05%) area of Malakand Division. The average incidence of virus was recorded 8.83% and 8.46 % in Hazara and Malakand divisions respectively (Table 1).

Symptoms based Incidence of TRV

Highest incidence of TRV in the fields of Hazara division was calculated in Gojri (8.08%) and in Malakand division maximum was noted in Shangla top (11.62%) area these were the highest as compare to other areas of the divisions. The minimum incidence of TRV was (5.31 %) in Nawansher in Hazara and Shahpur (8.24 %) area of Malakand. The average incidence of Hazara and Malakand was recorded, 6.80 % was recorded for first one and 9.95 % for previous one (Table 2).

ELISA based Incidence of PMTV

The ELISA based incidence of PMTV in the potato tubers samples collected from both Malakand and Hazara Divisions (Table 2). On the basis of ELISA results highest incidence was recorded in Baffa (21.73%) area of Hazara while in Kalam (23.80%) area of Malakand division. ELISA based lowest incidence of Hazara was recorded in Ghandian (13.33%) and in Shangla top (17.64%) was recorded in Malakand division in comparison with other studied areas. The average incidence of PMTV reported in Hazara division was 17.34 % whereas average incidence of the virus in Malakand division was 21.36 %.

ELISA based Incidence of TRV

ELISA based incidence of TRV in Malakand and Hazara Division was demonstrated (Table 2). In Malakand Division highest incidence was calculated from Shangla Top area which was 17.39% and lowest incidence of 11.11% Shahpur. Highest incidence calculated in Hazara Division was 14.28% in the samples collected in Mangal, and minimum incidence was noticed in Ghandian which was 9.09%. The average incidence of TRV reported for Hazara division was 11.99 % while 13.71 % was the average incidence of the virus in Malakand division.

Table 1. Incidence of soil-borne viruses in major potato growing areas of Hazara and Malakand divisions on the basis of field symptoms

Divisions	Locations	Incidence of soil-borne viruses					
		PMTV			TRV		
		Total no. of plants in 4 m^2	Plants showing symptoms	% Incidence	Total no. of plants in 4 m^2	Plants showing symptoms	% Incidence
Hazara	Mangal	95	8	8.42	92	7	7.60
	Baldahri	83	7	8.43	96	6	6.25
	Nawansher	94	9	9.57	94	5	5.31
	Gojri	99	9	9.09	99	8	8.08
	Datta	84	7	8.33	92	7	7.60
	Ghandian	83	6	7.22	87	6	6.89
	Baffa	93	10	10.75	85	5	5.88
Average Incidence		90.14	8	8.83	92.14	6.28	6.80
Malakand	Mandian	85	7	8.23	85	9	10.58
	Fatehpur	92	7	7.60	87	9	10.34
	Bahrin	90	8	8.88	90	8	8.87
	Kalam	87	9	10.34	87	9	10.34
	Shangla Top	86	8	9.30	86	10	11.62
	Kotkay	85	6	7.05	85	9	10.58
	Shahpur	97	7	7.21	97	8	8.24
	Bilkani	88	8	9.09	88	8	9.09
Average Incidence		89.28	7.5	8.46	88.12	8.75	9.95

Table 2. Incidence of soil-borne viruses in major potato growing areas of Hazara and Malakand divisions on the basis of ELISA detection

Divisions	Locations	Incidence of soil-borne viruses on ELISA base					
		PMTV			TRV		
		no. of samples collected	ELISA positive samples	% Incidence	no. of samples collected	ELISA positive samples	% incidence
Hazara	Mangal	17	3	17.64	21	3	14.28
	Baldahri	19	4	21.05	18	2	11.11
	Nawansher	16	3	15.75	16	2	12.50
	Gojri	17	3	17.64	23	3	13.04
	Datta	14	2	14.28	17	2	11.76
	Ghandian	15	2	13.33	22	2	9.09
	Baffa	23	5	21.73	26	3	11.53
Average Incidence		17.28	3.14	17.34	20.42	2.42	11.90
Malakand	Mandian	19	4	21.05	16	2	12.50
	Fatehpur	18	4	22.22	19	3	15.75
	Bahrin	14	3	21.42	21	3	14.28
	Kalam	21	5	23.80	25	3	12.00
	Shangla Top	17	3	17.64	23	4	17.39
	Kotkay	20	4	20.00	22	3	13.63
	Shahpur	23	5	21.73	18	2	11.11
	Bilkani	13	3	23.07	23	3	13.04
Average Incidence		18.12	3.87	21.36	20.87	2.87	13.71

Interaction and association of Spongospora subterranea and PMTV

The Jaccard index of similarity (Table 3.) shows the association of *spongospora subterranea* and PMTV on the basis of ELISA test results that the average association found in Hazara for 226 samples was 27.0% and 24.4% in Malakand Division for 267 samples. In Hazara 31.8% association was recorded from Baffa which was highest association among all studied areas while the lowest association was noticed in Datta was 20.0%. Kalam (33.3%) was the highest association and Kotkay (14.2%) was the lowest association found in Malakand Division calculated through Jaccard Index.

Interaction and association of nematodes and TRV

The association of vector nematode and TRV on the basis of ELISA results calculated through Jaccard Index of similarity (Table 4.). Highest association was noticed in Malakand at Shangla Top (31.5%) and Gogri (12.5%) in Hazara Division in comparison with other areas. Lowest level of association was found at Ghandian and Baffa (7.6%) in Hazara Division and Mandian (13.3%) was noticed in Malakand Division. The Jaccard Index of similarity value for total 171 samples collected in Hazara was 8.94% which is lower than the values for total 217 samples collected in Malakand Division which was 20.7%.

Table 3. Interaction and association of *S. subterranea* and PMTV on the basis of ELISA test results and calculated using Jaccard index of similarity.

Division	Locality	No. of samples	PMTV/ S.s [a]				% Jaccard index
			+/+ (a)	+/- (b)	-/+ (c)	-/-	
Hazara	Mangal	32	4	2	7	19	30.7
	Baldhari	29	5	2	10	12	29.4
	Nawansher	31	4	1	11	15	25.0
	Gojri	35	4	2	9	20	26.6
	Datta	33	3	2	10	18	20.0
	Ghandian	28	3	1	9	15	23.1
	Baffa	38	7	2	13	16	31.8
Total		**226**	**30**	**12**	**69**	**115**	**27.0**
Malakand	Mandian	32	3	1	10	18	21.4
	Fatehpur	37	3	1	8	25	25.0
	Bahrin	31	4	2	11	14	23.5
	Kalam	36	7	2	12	15	33.3
	Shangla Top	34	3	2	11	18	18.7
	Kotkay	33	2	2	10	19	14.2
	Shahpur	35	4	1	11	19	25
	Bilkani	29	5	3	9	12	29.4
Total		**267**	**31**	**14**	**82**	**140**	**24.4**

[a] values showing positive (+) and negative (-) test results for *Potato mop top virus* and *Spongospora subterrane* %J =a/ a+b+c x 100

Table 4. Interaction and association of nematodes and TRV on the basis of ELISA test results and calculated using Jaccard index of similarity.

Division	Locality	No.of samples	PMTV/ S.s [a]				%Jaccard index
			+/+ (a)	+/- (b)	-/+ (c)	-/-	
Hazara	Mangal	28	2	2	13	11	11.7
	Baldhari	24	1	3	8	12	8.3
	Nawansher	29	2	3	11	13	12.5
	Gojri	21	1	1	8	11	10.0
	Datta	27	1	2	9	15	8.3
	Ghandian	19	1	2	10	7	7.6
	Baffa	23	1	2	10	10	7.6
Total		**171**	**9**	**15**	**73**	**68**	**8.94**
Malakand	Mandian	25	2	3	10	10	13.3
	Fatehpur	26	4	2	9	11	22.2
	Bahrin	26	3	2	12	9	17.6
	Kalam	26	3	2	13	8	16.6
	Shangla Top	29	6	3	10	10	31.5
	Kotkay	30	5	2	12	11	26.3
	Shahpur	28	3	1	11	13	20.0
	Bilkani	27	2	1	8	16	18.1
Total		**217**	**28**	**16**	**85**	**88**	**20.7**

[a] values showing positive (+) and negative (-) test results for *Tobacco Rattle virus* and *Nematodes.*
%J =a/ a+b+c x 100

Biological characterization of soil-borne viruses of potato: field study

The biological characterization of PMTV and TRV in field studies provided the information that both viruses and their vectors are prevalent in major potato growing areas of Hazara and Malakand Divisions. The characteristic symptoms of PMTV were observed in fields. The infected plants showed bright yellow blotches on leaves, shortening of internodes causing stunting, and distortion of leaves. The tubers of infected plants showing the characteristic symptoms of PMTV were also examined and in most of the cases internal spraing and malformed or misshapen tubers were found, while some of the tubers had cracking. And the characteristic symptoms of TRV were observed in fields are type of necrotic arcing (known as spraing, corky ringspot) in tuber flesh, small spots or necrosis and ring spots lesions. The foliage symptoms are, leaf chlorosis, yellow rings on leaves, distortion of stem, stunting and stem mottling, confined to one or few stems grown from infected tubers (Harrison and Robinson, 1982)

Biological characterization of soil-borne viruses of potato: Bait test studies

The results of the biological characterization of PMTV and TRV through bait test. The original host of both the viruses i.e. potato was used as a bait plant. The plants were checked each time at 7 days interval, after germination, for symptoms development. No symptoms were observed on leaves of the bait plants till the harvest. After harvest the tubers were assessed for PMTV and powdery scab lesions. The examination of the tubers showed necrotic arcs in the flesh of the tubers and root galls or powdery scab lesions on the surface. The control pots in which the sterilized soil was used showed no symptoms of PMTV and powdery scab. The harvested tubers were also assessed for TRV and nematode lesions. The examination of tubers was done for nematode lesions on the surface of tubers as well as roots. The control pots in which the sterilized soil was used showed no symptoms of TRV and nematodes.

Biological characterization of soil-borne viruses of potato: Screen house studies on indicator host

In the screen house studies in which the indicator plants *Nicotiana benthamiana, Nicotiana debneyi, Nicotiana rustica and Chenopodium amaranticolor* were mechanically inoculated from an infected host plant. The potentially virus containing material (taken from the bait plants) was crushed with as little water as possible in a mortar. Virus free indicator plants were lightly dusted with 600 carborundum powder, which during inoculation causes small wounds in epidermal cells and increases the chance of transmission. The suspension of material was rubbed on the indicator plants using a clean finger. The inoculated leaves were marked to distinguish between primary infected leaves and secondary systematic infection (Bos, 1983). By inoculating PMTV isolated form bait test plants the symptoms on *Nicotiana benthamiana* were necrotic spots and necrotic local lesions with mild and systemic mosaic. On *Nicotiana debneyi* gives symptoms chlorosis with mild mosaic and moderate mosaic while in *Nicotiana rustica* the symptoms were chlorosis and necrosis with moderate mosaic was observed while *Chenopodium amaranticolor* produced necrotic and chlorotic local lesions with mild mosaic**.** The isolates of TRV are inoculated on the indicator hosts and in reaction host plants give the symptoms on *Chenopodium amaranticolor* were necrotic and chlorotic local lesion with necrotic. *Nicotiana debneyi* shows chlorotic and necrotic lesions and little systematic mosaic. *Nicotiana rustica* gives necrotic and chlorotic spots, and some little chlorosis with systemic mosaic. The symptoms on *Nicotiana benthamiana* were line pattern rings along with mottle and necrosis also shows some mild and systemic mosaic on leaves.

Serological characterization of PMTV and TRV isolates from Hazara division

Serological characterizations of the PMTV and TRV in field isolates from Hazara were made through DAS-ELISA technique. The potato cv. Rocco grown in the infested soils collected from various locations in Hazara division. The absorbance value (at 405 nm) for PMTV form tubers, leaves and roots of infested plants was calculated. The result indicated that PMTV was prevalent in all the locations surveyed in Hazara. Comparatively higher absorbance value for PMTV was obtained in Baffa locality. For the serological characterization of TRV the susceptible potato cv. Desiree was grown in the infested soil. TRV was extracted by DAS-ELISA technique. The absorbance value (at 405 nm) for TRV from the tubers, leaves and roots were calculated. The result of absorbance value shows that TRV was also prevalent in all locations. The higher absorbance value of TRV was noticed in Mangal area in Hazara division.

Serological characterization of PMTV and TRV isolates Malakand division

Serological characterizations of the PMTV and TRV in field isolates from Malakand division were also made through DAS-ELISA. After performing ELISA of the infested tubers, leaves and roots of potato cv. Rocco which is susceptible to PMTV. The absorbance values were calculated (at 405 nm). The results indicated that in all the areas surveyed PMTV was prevalent. The maximum absorbance value calculated for PMTV was in Kalam areas. The potato cultivar Desiree susceptible to TRV was grown to extract the virus form Malakand division and calculate the absorbance value. ELISA of the tubers, leaves and root was performed and absorbance values (at 405 nm) were calculated. The higher absorbance value for TRV was obtained in Shangla top area and virus was prevalent in all areas surveyed in Malakand division.

Response of back indexing and infectivity assay techniques for PMTV and TRV

The data regarding number of local lesions produced by PMTV on the back indexing plant showed significant difference among the treatment means. (Table 5.) The highest number of local lesions was observed in T_7

(9.33%) while the minimum numbers of local lesions were observed in T_{12} (3.66%). In the same table data for number of local lesions produce on the back indexing plant by TRV showed significant difference among the treatment means. The highest number of local lesions was observed in T_1 (9.66%) while the minimum number of local lesions was observed in T_9 (4.33%).

Response of soil borne-viruses PMTV and TRV based on bait test experiment

The results concerning response of potato against PMTV based on bait test experiment are shown. (Table 6) On the basis of ELISA value the statistical analysis of the data showed significant results ($\alpha = 0.05$) among them. All plants were susceptible to PMTV isolates. On the basis of ELISA absorbance value the means were figured at 5 % level of significance. The highest absorbance value for PMTV was observed in T_{11} (1.17), followed by T_7 (1.14) while the minimum value for PMTV was observed in T_{10} (0.75). The control treatments showed negative results for PMTV. The results of potatoes reaction against TRV based on bait test experiment shown in Table 6. On the basis of ELISA value the statistical analysis of the data showed significant results ($\alpha = 0.05$) among them. All plants were susceptible to TRV isolates. On the basis of ELISA absorbance value the means were figured at 5 % level of significance. The highest absorbance value for TRV was observed in T_{12} (1.07), followed by T_4 and T_{13} (1.04) while the minimum value for TRV was observed in T_5 (0.71). The control treatments showed negative results for TRV.

Table 5. Response of back indexing and infectivity assay techniques for soil-borne viruses PMTV and TRV

Treatments	Mean No. of local lesions on back indexing plant (*Chenopodium amaranticolor*)	
	PMTV	TRV
T_1	9.0 a	9.6 a
T_2	8.0 ab	7.0 bcd
T_3	8.0 ab	5.6 defg
T_4	5. efg	8.3 ab
T_5	5.0 efg	6.0 def
T_6	5.0 efg	6.3 cde
T_7	9.3 a	5.0 efg
T_8	4.3 fg	6.6 cd
T_9	6.3 cde	4.3 g
T_{10}	6.0 cde	4.6 fg
T_{11}	8.0 ab	6.6 cd
T_{12}	3.6 g	9.3 a
T_{13}	5.3 def	7.6 bc
T_{14}	6.6 bcd	5.6 defg
T_{15}	8.6 a	4.6 fg
T_0	0.0 h	0.0 h

Means followed by same letters do not differ significantly at P = 0.05. Each value is a mean of 3 observations
LSD of PMTV: (α=0.05) = 1.51 LSD of TRV: (α=0.05) =1.55

The present study reports the incidence and distribution of major soil-borne viruses (PMTV and TRV) infecting potato crop in Hazara and Malakand divisions and characterization of prevalent virus isolates. The incidence of PMTV and TRV evaluated on the basis of characteristic symptoms was lower than the incidence of the viruses detected through DAS-ELISA. The possible reason for such differences is that both the viruses (PMTV and TRV) are soil-borne and transmitted through *S.subterranea* and *Trichodoridae* nematodes, respectively. PMTV and TRV exhibits symptoms upon primary infection which could not be exhibited on above ground parts of the potato plant and hence, these plants look apparent healthy, while symptom-less infection could be detected through DAS-ELISA. Similar experiences were reported by various researches elsewhere while working on soil-borne viruses (Harrison and Jone, 1970)

The studies under discussion indicated that PMTV and TRV, both are quite prevalent in major potato growing areas of the Khyber Pakhtunkhwa (Hazara and Malakand). This is due to long prevalence and persistence of their vector, *S. subterranea* and the vector nematodes in the region. *S.subterranea* was reported in the areas in 1996 (Ahmed *et al*., 1996) but viruliferous nature of the plasmodiophorid vector was not known until recent investigation (M. Arif, unpublished). Association of TRV and its vector was not determined before; it may be the first report of TRV in the region. Another reason for the prevalence of soil-borne viruses and their vectors in this region was that the imports of seed tubers from various parts of the country and abroad without proper check and quarantine processes. This may be the reason that the virus available in seed tuber and soil infested in the vectors. Therefore, association of the virus with vector could acquire and become virulifeours. Similar evidence for acquisition and transmission of PMTV by *S. subterranea* was reported by Arif (1995) by conducting experiment on PMTV and *S. subterranea in vitro* and *in vivo*. Aviruliferous *S. subtrranea* became

viruliferous if plant already infected with PMTV. During division of plasmodia into zoosporangium, some of zoosporangia became infected and persist inside cystorus (cytosori) for longtime.

Table 6. Response of soil-borne viruses to bait test experiment

Treatments	ELISA value*	
	PMTV	TRV
T_1	1.07 abc	1.03 ab
T_2	0.91 def	0.93 ab
T_3	1.01 cd	0.87 ab
T_4	0.86 fgh	1.04 a
T_5	1.06 abc	0.71 b
T_6	0.88efg	1.02 a
T_7	1.14 ab	0.79 ab
T_8	1.05 abc	0.89 ab
T_9	1.00 cde	0.90 ab
T_{10}	0.75 h	0.96 ab
T_{11}	1.17 a	0.89 ab
T_{12}	1.03 bcd	1.07 a
T_{13}	0.77 gh	1.04 a
T_{14}	1.01 bcd	0.82 ab
T_{15}	1.09 abc	0.92 ab
T_0	0.31 i	0.27 c

Means followed by same letters do not differ significantly at P = 0.05. Each value is a mean of 3 observations
LSD for PMTV: (α=0.05) =0.1247 LSD for TRV: (α=0.05) =0.3003

Bait test supplemented ELISA and back indexing of sap from baited plant (root) successfully detected both viruses. However, detailed studies will be required to established variability among isolate, (if any) extending the area and by applying molecular techniques. Coat protein sequence analysis of the prevalent isolates of will generate data and comparisons with isolates elsewhere could helps to solve the issue. Therefore, more work is needed to amplify coat protein using RT-PCR and comparative analysis with isolate elsewhere in the world.

CONCLUSIONS AND RECOMMENDATIONS

Soil-borne viruses (PMTV and TRV) were prevalent in major potato growing areas of Hazara and Malakand divisions. Soil-bait test supplemented with DAS-ELISA, and back indexing of baited plant root to diagnostic hosts, successfully detected PMTV and TRV in fields.

The virus-free and certified seed tubers in non-infested field could help to minimize the spread of the soil-borne viruses. Detailed study is required to determine variability among isolates of soil-borne viruses on the basis of molecular properties.

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