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Attaullah
Department of Plant Pathology,
The University of Agriculture
Peshawar, Pakistan

Muhammad Arif
Department of Plant Pathology,
The University of Agriculture
Peshawar, Pakistan

Prevalence of major aphid and soil borne viruses infecting potato crop in North Western Pakistan

Attaullah and Muhammad Arif

Abstract

Prevalence of major aphid and soil borne viruses infecting potato crop in North Western Pakistan was evaluated during spring and summer crop in 2012-2013. On the basis of field symptoms the average incidence of Potato leaf roll virus (PLRV) ranged from 3.89% (Swat) to 46.66% (Swat), Potato Virus Y (PVY) ranged from 67.04% (Shangla) to 78.95% (Swat) and Potato mop virus (PMTV) ranged from 2.44% (Shangla) to 9.07% (Swat). On the basis of ELISA test average incidence of PLRV was ranged from 8.6% to 44.3%, PVY ranged from 67.0% to 78.9% and PMTV ranged from 2.44% to 9.07%. Field symptoms necrosis, leaf rolling and yellowing and V-shaped chlorosis were shown by PVY, PLRV and PMTV respectively. Field symptoms and serological studies revealed that PLRV, PVY and PMTV were the major viral diseases of potato in the areas surveyed.

Keywords: Potato, PLRV, PVY, PMTV, TRV Pakistan

1. Introduction

Potato is the most important crop of Pakistan and losses caused by viruses are up to 83% [1]. Potato virus Y (PVY) and Potato leaf roll virus (PLRV) have continued to be the most damaging and wide spread aphid borne viruses of potato crop [2]. These viruses decrease yield quantity and quality [3]. The incidence 15-65% of PLRV was reported from throughout the country [4] with yield losses recorded up to 90% [5] and losses caused by PVY in Pakistan have been estimated from 40-70% [4]. Soil borne virus such as *Potato mop top virus* (PMTV) is found nearly in all the seed potato growing areas of the country and in most parts of the Khyber Pakhtunkhwa. Its incidence has been reported on the basis of symptoms and considered to be serious threat to potato seed industry in Pakistan [6]. *Tobacco rattle virus* (TRV) has a widespread distribution [7].

Potato leaf roll virus belongs to genus *Polerovirus* and has a great importance in the history of potato and perhaps the beginning of potato viruses [8]. The primary infection caused by PLRV induces darkening of the vascular bundles and net necrosis in tubers. PLRV infected potato seeds cause symptoms of secondary infection which include rolling, reddening or yellowing of leaves and stunting of plant [9].

PVY is another major aphid borne virus belong to *Potyvirus* [8] and one of the most important pathogens of pepper, potato, tobacco and tomato crops. The virus is transmitted by many aphid species in a non-persistent manner including *Myzus persicae* (Sulzer) by seed and by sap inoculation in various experiments [10]. PVY infections can induce necrotic symptoms and affect both quantity and quality of the produce [11]. It is also known as severe mosaic virus in potato and causes mild to severe mottling or yellowing of the leaflets, necrosis, leaf dropping and early death of infected potatoes with yield losses up to 80% [12]. Three main groups (PVY^O, PVY^N and PVY^C) and in two subgroups (PVY^{NTN} and PVY^{N-W}) in PVY^N are found in PVY strain isolates on the basis of their capacity to induce necrotic symptoms on *Nicotiana tabacum* and their serological and molecular properties [13].

Potato mop top virus (PMTV) belongs to soilborne viruses gained critical importance due to unavailability of host resistance and perpetuation in tuber which is transmitted by *Spongospora subterranea* f. sp. subterranea (Wallr.) Lagerth, the soil-borne plasmodiophorid vector [14]. PMTV is the type species of genus Pomovirus, family Virgaviridae [8] has fragile rod-shaped particle with tri-partite single-stranded RNA genome [15]. It may cause both qualitative and quantitative loss [16, 17]. The symptomless tubers with latent infection can spread this virus because the incidence of symptoms in the same variety within the same field can vary from season to season [18].

Correspondence
Attaullah
Department of Plant Pathology,
The University of Agriculture
Peshawar, Pakistan

Different viruses such as PMTV and TRV can cause the necrotic symptoms of potato tubers [17, 10].

The infection of these viruses in susceptible potato cultivars cause spraing symptoms i.e internal rings and brown arcs in the tuber and rings and necrotic arcs on the surface of tubers [19].

Tobacco rattle virus (TRV) is another major soilborne virus which affects severely tuber quality and causes spraing symptoms in potatoes [20]. The affected tubers destroy the sale potential of the crop for human consumption and industrial use [21]. This disease is also known as corky ringspot disease in North America [22]. This virus is widely distributed in North America and Europe [23, 24]. Nematodes belong to *trichodorus* and *paratrachodorus species* of family *Trichodoridae*, transmitting TRV in potato crop which can survive and remain infective within the nematode for years [25].

The unavailability of quality virus free seed potatoes, presence of vector and negligence in quarantine regulations,

the incidence and distribution of potato viruses is increasing day by day. The study was conducted to diagnose and confirm these viruses with the objective to save the potato crop of the area.

2. Material and Methods

2.1 Field surveys and sampling

Three comprehensive surveys were conducted with one month interval of the major potato growing areas of northern districts of Khyber Pakhtunkhwa during spring (December to May) and summer crop (March-May to July-September) in 2012-2013 to assess the incidence and distribution of major aphid borne viruses (PLRV and PVY) and soil-borne viruses (PMTV and TRV). The areas under survey were divided into seven different zones. Each zone was further divided into three locations and each location was further divided into sites/fields (Table 1).

Table 1: List of major potato growing areas of northern parts of Khyber Pakhtunkhwa for surveys and assessment of major viruses of potato

S. No	Zone	Direction	Altitudes (ft)	Locations
01	Pir Khana (Shangla)	North	6000	Ganshal, Olander, Shonial
02	Shangla Hill (Shangla)	North	7000	Shanglatop, Kotkay, Machar
03	Miandam (Swat)	North west	6800	Miandam, Jokhtai, Senay
04	Kalam North (Swat)	North west	6800	KSES, Mateltan, Ushu
05	Kalam East (Swat)	North west	7500	Kalam, Utror, Gabral
06	Mansehra	North East	5040	Bajna, Baffa, Nokot
07	Abbotabad	North East	5040	Tandachuwa, Sheikhubanda, Jogian

During surveys at each location equal number of plant samples was selected in each field. Disease incidence was calculated according to % disease incidence formula [26].

$$\text{Percent incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

In each field plants were sampled in unit area. The values of all random spots (per field) were averaged together. The key used to calculate incidence of aphid borne viruses and soil borne viruses on the basis of characteristics symptoms is given in Table 2. Three single leaflets were taken from top, middle and bottom of the plant for each single sample. Tubers at harvest were washed and examined for scab and superficial spraing symptoms. Samples of tubers were also collected from each site of the selected field and were stored at 4-8 °C. Each of all these samples was placed in polythene sample bag separately and was labeled properly to indicate serial S.No, suspected viral problem, date of collection and location. These samples were brought to Department of Plant Pathology, The University of Agriculture Peshawar, and were stored at 4 °C in Plant Virology Laboratory for further

processing.

2.2 Das-Elisa

Samples collected during survey were tested through ELISA [27]. The procedure involved the following steps; ELISA plate was loaded with coating antibodies already diluted in coating buffer and incubated at 37 °C for 4 hours. With washing buffer the plates were washed and dried by paper towel. Then plant sample (100µl) extracted in extraction buffer by homogenizing through pestle and mortar was loaded. Positive and negative control (100µl) was also loaded and incubated at 37 °C for 4 hours. Plates were washed and dried with paper towel. Conjugated antibodies (100µl) were added in each well already diluted in conjugate buffer. Plates were again incubated for 37 °C for 1 hour. Plates were washed four times after incubation to make sure that all unwanted conjugates from the wells have been removed. Substrate was prepared (100µl) in substrate buffer, loaded and incubated in the dark at room temperature for half of an hour and reaction was observed for the development of yellow colour visually which shows the presence of a virus. The reaction was stopped by adding NaOH (50µl) to each well.

Table 2: Key used to assess the aphid borne viruses and soil borne viruses in the field on the basis of characteristic symptoms.

Disease	Pathogen	Characteristic foliage symptoms
Potato leaf roll*	Potato leaf roll virus	Primary infection: pallor or reddening of leaf tips of potato plants which may roll and become erect. Secondary symptoms: Plants grown from infected potato tubers include stunting of shoot and leaflets rolling upwards starting with the oldest leaves [28].
Potato mosaic *	Potato virus Y	Severe mosaic, mild to severe mottling or yellowing of the leaflets and necrosis
Potato mop top**	Potato mop top virus	Pale green chevrons (V-shaped marking) and bright yellow blotches on leaves Mottling or shortening of internodes (mop top) Brown or necrotic arcs on tubers (spriang) Scabs, cankers on tubers and galls on roots or stolons
Tobacco rattle**	Tobacco rattle virus	Mosaic pattern on leaves, Deformed stalk, Short petioles, Stem mottling Dark stripes and spots within and outside potato-tuber Stunted, abbreviated or "stubby" appearing root system

*[12, 11] ** [28, 16, 7]

3. Results

3.1 Incidence and distribution of aphid borne (PLRV and PVY) and soil borne (PMTV and TRV) viruses on the

basis of field symptoms

The incidence and distribution of these viruses are shown in Table 3 that average

Table 3: Incidence and distribution of aphid borne viruses (PLRV and PVY) and soil borne viruses (PMTV and TRV) on the basis of field symptoms

Zone	Locality	Locality incidence (%)				Zone incidence (%)			
		PLRV	PVY	PMTV	TRV	PLRV	PVY	PMTV	TRV
Pir Khana (Shangla)	Ganshal	13.00	51.00	00.00	0	16.33	52.55	06.00	0
	Olander	26.66	66.66	10.00	0				
	Shonia	10.00	40.00	08.00	0				
Shangla Hill Shangla)	Shanglatop	33.33	51.85	11.11	0	28.88	49.99	09.13	0
	Kotkay	43.33	36.36	04.54	0				
	Machar	10.00	61.76	11.76	0				
Miandam (Swat)	Miandam	4.34	69.56	15.21	0	3.89	69.30	15.22	0
	Jokhtai	4.00	52.00	13.79	0				
	Senay	3.33	86.36	16.66	0				
Kalam North (Swat)	KSES	46.66	77.77	13.33	0	46.66	58.25	13.66	0
	Mateltan	36.66	50.00	13.75	0				
	Ushu	56.66	47.00	13.00	0				
Kalam East (Swat)	Kalam	20.00	53.33	06.00	0	27.77	47.77	5.90	0
	Utror	30.00	50.00	05.71	0				
	Gabral	33.33	40.00	06.00	0				
Mansehra	Bajna	40.00	29.41	14.70	0	26.66	31.93	12.73	0
	Baffa	30.00	50.00	08.75	0				
	Nokot	10.00	16.39	14.75	0				
Abbotabad	Tandachuwa	10.00	21.73	08.69	0	06.44	52.26	9.92	0
	Sheikhulbandi	5.17	51.72	12.00	0				
	Jogian	4.16	83.33	09.09	0				

KSES: Kalam Summer Experimental Station

Incidence of PLRV ranged from 3.89 – 46.66 %. The maximum incidence was recorded in Kalam North (46.66 %) at Ushu locality (56.66%) and minimum incidence was recorded in Miandam zone (3.89 %) at Senay locality (3.33%) whereas, average incidence of PVY ranged from 31.93 - 69.30 %. The maximum incidence was recorded in Miandam zone (69.30 %) in District Swat and at Senay locality (86.36%) in the same zone and minimum incidence was recorded in Mansehra zone (31.93%) in District Mansehra and at Tandachuwa locality in District Abbottabad (21.73%). The average incidence of PMTV ranged from 6.00 – 15.22 %. The maximum incidence was found in the zone of Miandam, District Swat (15.22 %) at locality Senay (16.66 %) in the same zone and minimum incidence was recorded in Pir Khana zone in District Shangla (6.00%) at locality Ganshal of the same zone. Whereas, no symptoms of TRV was found in all the zones surveyed. Fig. 1-4 showed the field symptoms of PLRV, PVY and PMTV respectively.



Fig. 1: Field symptoms of PLRV collected during survey in Shangla hills area



Fig. 2: Field symptoms of PVY infected leaves collected during survey in Shangla hills area



Fig. 3: Field symptoms of PMTV infected leaves collected during survey in Abbotabad



Fig. 4: Scab symptoms on potato tubers caused by *Spongospora subterranean*, vector of PMTV

3.2 Incidence and distribution of aphid borne viruses (PLRV and PVY) and soil borne (PMTV and TRV) on the basis of ELISA test

The incidence of aphid borne viruses (PLRV and PVY) and soil borne (PMTV and TRV) on the basis of ELISA is

presented in the Table 4 which showed that average incidence of PLRV was ranged from 8.66 – 44.33 %. The maximum incidence was recorded in Kalam North (44.33 %) at Matiltan locality (50.00%) and minimum incidence was recorded in Miandam zone (8.66 %) at Jokhtai locality (3.00%). Whereas, average incidence of PVY ranged from (67.04 - 78.95 %). The maximum incidence was recorded in Kalam East zone (78.95%) in District Swat and at Senay locality (86.36%) in the Miandam zone in the same District and minimum incidence was recorded in Shangla Hill zone (67.04%) in District Shangla and at Nokot locality in District Mansehra (58.02). The average incidence of PMTV ranged from 2.44 - 9.07%. The maximum incidence was recorded in Kalam North zone (9.07%) in District Swat and at locality KSES (13.33%) in the same zone and minimum incidence was recorded in Pir Khana zone (2.44%) in District Shangla at Ganshal locality of the same zone (00.00). Whereas TRV showed zero incidence on the basis of ELISA test.

Table 4: Incidence and distribution of aphid borne viruses (PLRV and PVY) and soil borne viruses (PMTV and TRV) on the basis of ELISA test

Zone	Locality	Locality incidence (%)				Zone incidence (%)			
		PLRV	PVY	PMTV	TRV	PLRV	PVY	PMTV	TRV
Pir Khana (Shangla)	Ganshal	10.00	62.85	00.00	0	14.33	74.06	2.44	0
	Olander	30.00	83.33	03.33	0				
	Shonial	13.00	76.00	04.00	0				
Shangla Hill (Shangla)	Shanglatop	36.66	66.66	07.40	0	23.55	67.04	4.42	0
	Kotkay	30.00	72.72	00.00	0				
	Machar	04.00	61.76	05.88	0				
Miandam (Swat)	Miandam	10.00	78.26	00.00	0	8.66	76.20	4.18	0
	Jokhtai	03.00	64.00	08.00	0				
	Senay	13.00	86.36	04.54	0				
Kalam North (Swat)	KSES	43.33	73.33	13.33	0	44.33	76.36	9.27	0
	Mateltan	50.00	83.75	07.50	0				
	Ushu	40.00	72.00	07.00	0				
Kalam East (Swat)	Kalam	30.00	78.00	06.00	0	28.66	78.95	6.85	0
	Utror	26.00	72.85	08.57	0				
	Gabral	30.00	86.00	06.00	0				
Mansehra	Bajna	40.00	76.47	08.82	0	26.33	73.58	8.84	0
	Baffa	20.00	86.25	06.25	0				
	Nokot	20.00	58.02	11.47	0				
Abbotabad	Tandachuwa	13.00	80.43	10.86	0	14.33	73.98	8.93	0
	Sheikhulbandi	10.00	70.68	03.44	0				
	Jogian	20.00	70.83	12.50	0				

KSES: Kalam Summer Experimental Station

4. Discussion

Potato cultivation is major crop in northern parts of Khyber Pakhtunkhwa. The survey of this area was aimed to know the prevalence of the aphid borne (PLRV and PVY) and soil borne viruses (PMTV and TRV). Farmers of the northern parts of Khyber Pakhtunkhwa use smallest tubers as seed potatoes or those having no value. Such practice would lead to the spread of viral infections in the same area of potato cultivation as well as virus spread from one region to another. Virus infections seriously affect potatoes when some of the domestic potato seeds were screened after harvest.

The results obtained from the present field and laboratory study showed that PLRV, PVY and PMTV can reduce potato production in northern parts of Khyber Pakhtunkhwa. ELISA results showed higher infection rates in testing samples than visual inspection. The present results are in line agreement with Batool *et al.* findings [29] Where he reported that symptomology is an initial step to disease diagnosis and is not a reliable method for confirmation of viruses because symptoms caused by many factors such as time of infection,

environmental conditions, virus strain, insect sucking, nutrition deficiency, host genotype and growth stage etc. The differences in the incidence in different fields probably due to migratory aphid activity and differences in seed sources. Usually for propagation the farmers select the smallest tubers they harvest. Hane and Hamm [30] reported in his findings that PVY infection may increase number of small tubers which can lead to selection of the main source of survival of the virus and infected tubers. Furthermore, in the spread of aphid-borne viruses and in differences recorded in virus incidences the amount of initial inoculum has a vital role [2]. Infected volunteer plants and infected spring crops are sources of inoculum for the later planted autumn crops [31]. Secondary infection play important role in the epidemiology of the virus particularly in case of PVY [12].

Serological results showed the presence of PLRV and PVY in aphid borne viruses and PMTV in soil borne viruses in seven different zones of potato in the northern parts of Khyber Pakhtunkhwa. ELISA tests are more reliable for identification and detection of viruses. This is the reason that ELISA

confirmed the potato viruses in all field samples surveyed. Our results regarding the incidence of aphid borne viruses (PLRV and PVY) on the basis of ELISA test are in agreement with the results reported by Ali *et al* [32].

Mostly the current results regarding the visual evaluation of PMTV coincides with results of Calvert *et al* [33] Where they reported that during the survey no potato tuber showed PMTV-like spraing symptoms. Probably, environmental conditions, PMTV strain, cultivar resistance or an interaction of one or more of these factors suppressed the development of these symptoms. Similarly our results with respect to foliar infection also are in lines with Calvert *et al* [33] where about 82% of the foliar samples for PMTV asymptomatic were showed positive test and several foliar samples tested negative with ELISA with symptoms typical of PMTV.

In present study detection of PMTV in tubers by ELISA was greater than in foliar tissue coinciding with former results reported by Sokmen *et al* [34]. Interestingly, the detection of PMTV in tubers and in leaf tissue was not coincident (i.e. tested positive for PMTV both in leaves and tubers few samples showed positive results simultaneously). Such inconsistencies may be due to the ELISA technique limitations or due to uneven distribution of the virus. These results indicated the need for testing of both tissues to avoid underestimation of virus incidence in the fields during surveying.

The survey conducted clearly showed that PMTV infection occurred in all of the major growing areas of the northern parts of Khyber Pakhtunkhwa with no field infected with TRV. The incidence of PMTV based on the field symptoms indicated higher percent disease incidence in Miandam, District Swat. It may be due to higher humidity and longer period of soil wetness which are favorable for the development of vector and virus [35]. Furthermore, mostly growers use uncertified seed and foliar symptoms in the field suggested that seed potato was already infected by PMTV [28]. However, the incidence based on field symptoms can mislead the actual incidence because of the possibility of latent infection.

The incidence of PMTV based on ELISA showed higher incidence as compared to the incidence based on field symptoms. The variability in the incidence amongst the individual field and in localities may be due to the difference in climate, altitude and soil type. The higher incidence in some of the localities may be due to high average rainfall and high elevation. Similar findings were also reported by Montero-Austa *et al* [36] from Peru and Costa Rica where the incidence of PMTV in fields located at higher elevation was higher. Our results regarding incidence based on spraing symptoms also showed symptomless infection of PMTV in potato tubers. These results are in agreement with the previous findings reported by Sokemen *et al* [34]. Mostly the tubers infected with PMTV were not affected by spraing symptoms showing that symptomatic tuber infection may not be more wide spread than latent infection. These results are in contrast to the findings of Davey [37] who reported that two third of the tubers infected with PMTV were not affected by spraing symptoms. This may be due to susceptibility of the cultivar, environmental conditions and the PMTV strain that favored the formation of spraing.

5. Conclusion

PVY and PMTV are the major viruses infecting potato crop in northern parts of Khyber Pakhtunkhwa and serological method is the best one, reliable, authentic, less time

consuming and need no laborer for diagnosis and investigation.

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