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Incidence of six potato viruses in spring, summer and autumn potato crops of the North West Frontier Province of Pakistan

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Abstract. Spring, summer and autumn potato crops, grown in three different ecological zones (Peshawar, Swat and Hazara districts, respectively) in the North West Frontier Province of Pakistan were surveyed for potato viruses during 1999. A total of 1338 samples from 76 fields was tested by dot-immunobinding assay using antisera to six different viruses. Two major aphid-borne viruses, *Potato leaf roll virus* and *Potato virus Y* (PVY), were frequently detected in potato crops with incidences ranging from 0–14.7% in spring crops, 1.8–45.5% in summer crops, and 0–71.0% in autumn crops. Three other viruses, *Potato virus A*, *Potato virus S* and *Potato virus X* (PVX), were also detected but they were primarily limited to the summer crop, with incidences of 0–5.5%, 0–3.4% and 1.4–8.3%, respectively. PVX was also noted in the spring crop, with an incidence of 0–3.2%. None of the samples tested positive for *Potato virus M*. PVY was detected in 6–52% of potato tubers collected from potato growers in the Peshawar district.

Introduction

Potato (*Solanum tuberosum*), a native of South America, is the fourth most important food crop worldwide (Harton 1987). It has played a significant role in the industrial revolution of many European countries and has the potential to feed millions in other parts of the world due to its wide adaptability and high yield. Potato is affected by more than 27 viruses (Jayasinghe and Salazar 1998) but the major aphid-borne viruses, *Potato leaf roll virus* (PLRV; genus *Polerovirus*) and *Potato virus Y* (PVY; genus *Potyvirus*) are the most economically important worldwide.

PLRV in potatoes was first reported in Germany and Denmark (Rich 1983; Barker and Waterhouse 1999) and causes rolling and yellowing of the leaves that later become stiff, dry, leathery, crisp and papery to touch. PLRV is a phloem-limited virus and is transmitted in a persistent circulative manner by more than ten aphid species (Harrison 1958; Brigneti and Jayasinghe 1992). The green peach aphid (*Myzus persicae*) is the most important and efficient vector followed by the potato aphid (*Macrosiphum euphorbiae*) (MacCarthy 1954; Hooker 1981). Yield reduction from PLRV infection can be as high as 80–95% in susceptible cultivars (Matthews 1982; Barker and Waterhouse 1999).

PVY was first reported in the United Kingdom (Brunt *et al*. 1997) and causes necrosis, mild to severe mottling or yellowing of the leaflets, leaf dropping and sometimes premature death of infected potatoes (Khurana and Garg 1998). PVY is transmitted in a non-persistent manner by more than five aphid species, including *M. persicae*, by seed and experimentally by sap inoculation (Brunt *et al*. 1997). PVY infection can depress tuber yield by up to 80% depending on the strain of virus and the cultivar of potato (Khurana and Garg 1998).

In Pakistan, potato crops are grown on 550 000 ha with a total production of 5.5 million tonnes annually (Anon. 1998). The average yield of potatoes in Pakistan (10 tonnes/ha) is well below the world average (Anon. 1998). PLRV and PVY have been recorded in Pakistan (Mughal and Khalid 1985; Mughal *et al*. 1988) but little is known about these viruses in the North West Frontier Province (NWFP) where potato is cropped three times a year in spring, summer and autumn. The objective of the work reported here was to survey potato crops grown in NWFP for the presence of viruses, particularly PLRV and PYV, and to determine their distribution in the major growing areas.

Methods

Antisera sources

All antisera to PLRV, *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato virus X* (PVX) and PVY used in this study (Table 1) were from the collection of the Department of Plant Pathology, NWFP Agricultural University, Peshawar. Goat anti-rabbit IgG was obtained commercially (Sigma).

Survey and sampling

Potato crops (spring, summer and autumn) were surveyed in three different potato growing districts of NWFP during 1999. The spring crop is grown from February to May in the Peshawar district, the summer crop from May to August in the Swat district and the autumn crop from September to December in the Hazara and Peshawar districts. The Hazara (altitude 1680 m) and Swat (altitude 1170 m) districts are

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Table 1. Incidence of six viruses in spring, summer and autumn potato crops as detected by dot immunobinding assay

Locations	No. fields surveyed	No. samples tested	Incidence of infection (%)									
			Crop							Tuber		
			PLRV	PVA	PVM	PVS	PVX	PVY	PLRV + PVY	No. seed lots	PLRV	PVY
Spring crop												
Peshawar district												
AUPEF[A]	1	22	0	0	0	0	0	0	0	—[D]	—	—
Azakhel	2	25	0	0	0	0	0	12.0	0	30[E]	0	10
Dagai	3	30	0	0	0	0	0	13.3	0	48	0	16.6
Dagbaisud	6	92	0	0	0	0	3.2	5.4	0	48	0	6.3
Pabbi	3	34	0	0	0	0	0	14.7	0	48	0	52.1
Mean			0	0	0	0	0.64	9.1	0		0	21.3
Summer crop												
Swat district												
KSES[B]	2	36	8.3	5.5	0	2.7	8.3	13.8	0	—	—	—
Kalam	6	141	18.4	0	0	0	6.3	41.8	4.2	—	—	—
Ushu	10	202	9.9	0	0	3.4	1.4	45.5	4.9	—	—	—
Utror	7	165	1.8	0	0	0	1.8	24.8	2.4	—	—	—
Mean			9.6	1.4	0	1.5	4.5	31.5	2.9	—	—	—
Autumn crop												
Hazara district												
Chunakari	4	61	0	0	0	0	0	39.3	4.9	—	—	—
Kalapul	4	68	1.4	0	0	0	0	52.9	4.4	—	—	—
PRSA[C]	3	52	1.9	0	0	0	0	19.2	0	—	—	—
Rush	7	100	11.0	0	0	0	0	50.0	2.0	—	—	—
Mean			3.6	0	0	0	0	40.4	2.8	—	—	—
Peshawar district												
Azakhel	4	72	0	0	0	0	0	62.5	0	—	—	—
Dagai	4	76	0	0	0	0	0	71.0	0	—	—	—
Dagbaisud	5	80	0	0	0	0	0	66.2	0	—	—	—
Pabbi	5	82	0	0	0	0	0	68.2	0	—	—	—
Mean			0	0	0	0	0	67.0	0	—	—	—

[A]Agricultural University Peshawar Experiment Farm (AUPEF). [B]Kalam Summer Experiment Station (KSES). [C]Potato Research Station Abbottabad (PRSA). [D]—, not tested. [E]Mother potato tubers from farmers seed stock.

in the hilly region and the Peshawar district (altitude 300 m) is on the plain. These three districts are each separated by more than 100 km.

At each location, 10–24 samples per field were collected from both symptomatic and non-symptomatic plants by a random crop sampling procedure (Barnett 1986). Samples were placed separately in plastic bags and kept at 4°C until testing. Visual assessment was made by recording symptoms of the viruses in every field. In the Peshawar district, potato tubers left over after sowing were also collected from farmers during the survey. Weed, volunteer or neighbouring crop species adjacent to potato fields were observed and only symptomatic plants were sampled.

Sample preparation

Samples of leaf or tuber (1.0 g) were crushed individually in plastic bags with 1 mL of PBS buffer and the extract then centrifuged at 12 000 *g* for 3 min. Aliquots (1 µL) of supernatant were dotted onto a nitrocellulose membrane (Sigma), air-dried and either analysed immediately or stored at 4°C.

Detection by dot immunobinding assay (DIBA)

DIBA was carried out as described by Ali and Randles (1997) with the following modifications. Membranes dotted with leaf samples were blocked for 1 h in PBS buffer containing healthy, potato-leaf extract (1 g/9 mL) whereas membranes dotted with tuber samples were blocked in PBS buffer containing healthy, potato-tuber extract (1 g/9 mL). Sap from known PLRV- and PVY-infected plants was dotted onto the membrane at dilutions of 1/10, 1/100 and 1/1000 for use as positive controls.

Results

Sensitivity of DIBA

To determine the relative sensitivity of DIBA for detecting PLRV and PVY, ten-fold dilution series of infected plant extracts were tested. The limit of detection for PLRV was 1/100 whereas that for PVY was 1/1000. No non-specific reactions with healthy leaf or tuber extracts were observed.

Virus surveys

Results of virus surveys in spring, summer and autumn crops are shown in Table 1. In the spring crops of the

Peshawar district, 50% of potato plants showed mosaic, mottling, interveinal chlorotic spotting and leaf rolling. Rolling was mainly in the mature leaves of the plant. Of the 203 samples from five locations, none tested positive for PLRV, PVA, PVM or PVS, but PVY infection ranged from 0–14.7%. PVX was detected at one location (Dagbaisud) but infected only 3.2% of the plants.

In the summer crops of the Swat district, 20–70% of plants observed were symptomatic. In many fields, mosaic symptoms were mild to severe and were mostly observed in the early growth stage of the crop. In addition, chlorotic spotting, broader chlorosis and necrosis, puckering and upward rolling of the leaves were observed. A total of 544 samples was collected from four locations including Kalam Summer Experimental Research Station. Incidences of PLRV infection ranged from 1.8–18.4%, PVY infection from 13.8–45.5% and PVX infection from 1.4–8.3%. PVA (0–5.5%) and PVS (0–3.4%) infections were found at one and two sites, respectively. None of the samples tested positive for PVM. Mixed infections of PLRV and PVY were found at three locations (20 samples) but not at Kalam Summer Experimental Research Station (Table 1).

In the autumn potato crops, 30–40% of the plants from the Hazara district and 30–50% plants from the Peshawar district were symptomatic. Common symptoms were leaf mosaic, mottling and rolling. Less common were symptoms of chlorotic and necrotic leaf spotting, accompanied by stunting. At the four locations from the Hazara district, PLRV infection ranged from 0–11.0% and PVY from 19.2–52.9%. PVA, PVM, PVS and PVX were not detected in any sample. Mixed infections of PLRV and PVY were also recorded at three of the four locations. In the Peshawar district, PVY infection ranged from 62.5–71.0%. PLRV, PVA, PVM, PVS and PVX were not detected.

Incidence of PVY in potato seed

To determine the level of seed infection by PLRV and PVY, potato tubers collected from farmers during the surveys in the Peshawar district were tested by DIBA with PLRV and PVY antiserum. Of the 204 tubers from four locations, the incidence of PVY ranged from 6–52% (Table 1), but no tubers tested positive for PLRV.

Alternative hosts of PLRV and PVY in potato growing areas

Two common weeds (*Amaranthus* sp. and *Chenopodium* sp.) and volunteer or neighbouring chillies (*Capsicum frutescens*) and tomato (*Lycopersicon esculentum*) plants were recorded in, or adjacent to, potato fields during the surveys. All four species had symptoms of virus infection: *Amaranthus* sp. and *Chenopodium* sp. had chlorotic spots on the leaves, chilli plants had leaf interveinal chlorosis and tomato plants showed mottling symptoms. All four species tested positive for PVY by DIBA (Table 2). None tested positive for PLRV.

Discussion

Our survey results showed the presence of at least five viruses (PLRV, PVA, PVS, PVX and PVY) in spring, summer and autumn potato crops in three different ecological zones of the NWFP. Of these viruses, PVY was

Table 2. Detection of PLRV and PVY in alternative hosts by dot immunobinding assay

Locations	No. infected/No. tested							
	Amaranthus sp.		*Chenopodium* sp.		Chillies		Tomato	
	PLRV	PVY	PLRV	PVY	PLRV	PVY	PLRV	PVY
				Spring crop				
Peshawar district								
Azakhel	0/5	0/5	0/5	0/5	—[A]	—	—	—
Dagai	0/2	0/2	0/4	0/4	—	—	—	—
Dagbaisud	0/2	1/2	0/2	2/2	—	—	—	—
Pabbi	0/3	0/3	0/3	0/3		—	—	—
Dagbaisud	—	—	—	—	—	—	—	—
				Summer crop				
Swat district								
Kalam	0/3	2/3	0/4	4/4	0/6	6/6	0/3	3/3
Ushu	0/2	1/2	0/2	2/2	0/3	3/3	0/6	5/6
Utror	0/2	2/2	0/3	2/3	0/5	5/5	0/5	4/5
				Autumn crop				
Hazara district								
Chunakari	0/5	0/5	0/5	1/5	0/4	3/4	0/5	5/5
Kalapul	0/4	1/4	0/3	2/3	0/5	3/5	0/3	2/3

[A]—, not tested.

most widespread and occurred in 12 of the 13 locations surveyed. PLRV was next most widespread, occurring in 7 of the 13 locations. PVA, PVS and PVX were mostly a problem in the summer crop of the Swat district. Interestingly, no crops appeared to be infected with PVM.

PVY infection was found in nearly all potato crops but varied in incidence, possibly due to differences in seed sources and migratory aphid activity. For example, the incidence of PVY in potato tubers obtained from the summer crop of 1998 varied among the four locations. The incidence of PVY in the summer and autumn crops was higher than that in the tubers, suggesting that there had been significant secondary spread of the virus. Consistent with this observation, many weeds and volunteer chilli and tomato plants in the summer and autumn crops (Table 2) were infected with PVY and this infection probably originated from the potato crop. Infected tubers are considered to be the main mode of survival of PVY between potato crops in Pakistan (Khurana and Garg 1998).

During spring and autumn, no PLRV infection was recorded in the Peshawar district, although leaf rolling symptoms were observed on many potato plants. These results suggest that other abiotic or biotic factors can cause leaf rolling, and symptoms alone are inadequate for diagnosing PLRV infection (Jayasinghe and Salazar 1998). It is possible that we missed some cases of PLRV infection through lack of sensitivity of the DIBA. However, as both plants and tubers tested negative for PLRV, it is more likely that PLRV was rare or absent in the Peshawar district in that particular year.

Potato tubers are regularly circulated among the three districts in NWFP. For example, to plant the spring crop in the Peshawar district, most farmers used potato tubers produced from the previous summer crop grown in the hilly areas of the Swat district. Crops in the Swat district are in turn grown from tubers collected from the previous autumn potato crop of Punjab Province or from their own seed collected from the previous potato crop. With the autumn crop, farmers use the previous season autumn tubers of their own or those brought from the autumn crop of Punjab. In general, most of these seed tubers are uncertified and are a mixture of many different potato cultivars which may be infected with viruses.

The cultural practices adopted by the growers, conducive environmental conditions for epidemic development and lack of awareness by the growers of virus diseases are some of the major factors that contribute to the high incidence of these viruses, especially PVY. Among these, cultivation of uncertified potato seed, the presence of infected weed and volunteer potato and other solanaceous crop species, and overlapping potato crops are important factors contributing towards virus incidence and spread.

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