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## Prevalence of major potato viruses and aphid population dynamics in Punjab, India

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### Abstract

A survey was conducted over six major potato growing districts of Punjab during December of 2014 and 2015, respectively. Leaf samples of 190 potato plants showing various virus diseases like symptoms were collected during the survey for the two consecutive years. These leaf samples were crushed in extraction buffer and were exposed against antisera of seven major potato viruses viz. PVX, PVY<sup>n</sup>, PVY<sup>o/c</sup>, PVA, PVM, PVS and PAMV in DAS/TAS-ELISA. Study on various symptoms produced by these viruses on potato was done. Among the potato viruses, the highest incidence was recorded for PVX, 41.36 % and 89.47 % in the year 2014 and 2015, respectively. Maximum collection of infected potato plants were found to be co-infected with PVX and PVY<sup>n</sup> (73.68 %) in 2015 which exhibited rugose mosaic type of symptoms. Potato plants infected simultaneously with multiple potato viruses showed malformation of leaves, rosetting and severe stunting. The per cent viral disease incidence recorded was correlated with aphid populations observed over potato grown in these districts. The highest viral disease incidence was recorded in Hoshiarpur district, 17.47 % and 21.07 % for the year 2014 and 2015, respectively. This was due to relatively more abundance of aphid population in Hoshiarpur district among others. The incidence of PVX, PVY<sup>n</sup>, PVY<sup>o/c</sup>, PVA, PVM, PVS was higher during 2015 as compared to the year 2014 due to increase in mean aphid population during the potato growing seasons.

**Keywords:** Survey, ELISA and potato

### 1. Introduction

Potato is one of the most important *Rabi* season crops of Punjab with a production of 2222.1 metric tonnes over an area of 87.24 thousand hectares [3]. The major varieties grown in the state includes Kufri Pukhraj, Kufri Chandramukhi, Kufri Surya, Kufri Jyoti, Kufri Bahar, Kufri Ashoka, Kufri Badshah and Lady Rosetta. Viral diseases of potato are prevalent throughout India and are most severe in North-eastern plains where the population of aphid vector is high throughout the crop season. Major viruses infecting potato are *Potato virus X* (PVX), *Potato virus Y* (PVY), *Potato acuba mosaic virus* (PAMV), *Potato leaf roll virus* (PLRV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Potato virus A* (PVA), *Potato virus S* (PVS) and *Potato virus M* (PVM). Among these viruses, PVY causes yield loss up to 90 per cent. Again, potato as a host of CMV has been reported by [10] from India, [2] from Syria, recently by [11] and [6] from Punjab, India. Most of these viruses are transmitted non-persistently by aphids. Again, potato-cucurbit cropping system is widely practiced in Punjab. Hence, some of the viruses may be transmitted from potato to cucurbits by aphids.

As potatoes from Punjab are distributed throughout the country and are exported to other nations, it is important to carry out regular survey to investigate prevalence of different viruses and to check the threat of emergence of new viruses. Simultaneously, it is necessary to know the prevailing population dynamics of aphids. In this present investigation we carried out survey over major potato growing districts of Punjab to know the prevalence of different viruses on potato and to observe the viral disease incidence in different districts.

### 2. Materials and Methods

#### Survey and sample collection

Field visits were conducted to villages of major potato growing districts of Punjab viz. Patiala, Barnala, Ludhiana, Kapurthala, Jalandhar and Hoshiarpur. Random sampling was done as the area under cultivation of potato varies among these districts. Suspected potato leaf samples showing various viral diseases like symptoms were collected from 95 potato plants in random

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from the 24 fields of these districts each year during December of 2014 and 2015, respectively. During sample collection, various symptoms exhibited by the infected potato plants were observed and recorded to correlate with positive, negative samples and mixed infection. The per cent disease incidence was recorded randomly from five spots including four corners and one central patch of 1m<sup>2</sup> of one field at a particular location. The per cent disease incidence was calculated by counting the total number of plants and number of plants showing symptomatic viral infection using the formula given below:

$$\text{Viral Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

### Serological Detection

According to the protocols of [5], potato leaf samples collected from 190 plants were subjected to TAS-ELISA against the antibodies of PVY<sup>n</sup> and PVY<sup>o/c</sup>. The same potato leaf samples were subjected to DAS-ELISA against the antibodies of PVX, PVA, PVM, PVS and PAMV. The antisera provided within the Agdia ELISA kit were used to conduct the present experiment. ELISA plates were coated with 100 µl of coating buffer containing PVX, PVY, PVA, PVM, PVS and PAMV polyclonal capture antibody and were thereafter incubated overnight at 4°C. 100 µl of diluted plant sap extracts (sample to extraction buffer ratio of 1:8) was added to the wells after providing alternate washing with wash buffer and distilled water. This step was again followed by incubating the plates at 37°C for 2 hours and washing with wash buffer. Detection antibody and alkaline phosphate enzyme conjugated antibody (having strain specificity) of PVY<sup>n</sup> and PVY<sup>o/c</sup> were together diluted in conjugate ECI buffer. While only alkaline phosphate enzyme conjugated antibody was diluted in conjugate ECI buffer for PVX, PVA, PVM, PVS and PAMV. 100 µl of the antibody conjugate buffer solution was loaded into each well. The dilution ratio of the antibody to conjugate buffer was kept as 1:200 (according to Agdia protocols). After incubating the plates at 4 °C for overnight and alternate washing, the wells in the plates were loaded with 100 µl p-nitro-phenyl-phosphate substrate solution. Finally, plates were tightly covered with aluminium film and incubated for 60 minutes at room temperature in the dark. The colour changes were read visually and photometrically with ELISA Reader (Sunrise, Tecan, Austria) at 405 nm. Colour change in the wells indicated the presence of virus in leaf samples. The positive and negative controls were procured from ELISA kit provided by Agdia.

### Aphid population dynamics

Aphid population was recorded at every 7 days interval over potato plants during the months of November, December, January and February in 2014 and 2015, respectively at Patiala, Barnala, Ludhiana, Kapurthala, Jalandhar and Hoshiarpur districts of Punjab. One village from each district was selected as sites for recording aphid population dynamics on basis of area under highest potato cultivation and significantly contributing in the supply of table potatoes *viz.* Nabha of Patiala, Thulewal of Barnala, Plant Pathology Research Farm, PAU of Ludhiana, Kala Sanghian of Kapurthala, Pattar Kalan of Jalandhar and Phuglana of Hoshiarpur district, respectively. Enumeration of aphid population was done by following the standard method of counting aphids over 100 compound leaves in an area [12]. Aphids were counted randomly from one upper, one middle

and one lower compound leaf from each of 33 plants and one leaf of the 34<sup>th</sup> plant.

## 3. Results and Discussion

### Survey

On the basis of visual observation, the viral disease incidence during 2014 on potato varied from 8.89 per cent at Barnala to 17.47 per cent at Hoshiarpur district. The viral disease incidence was found to be more during the year 2015 ranging from 9.56 per cent at Barnala to 21.07% at Hoshiarpur district during the year 2015 (Figure 3). Thus in both the consecutive years the highest viral disease incidence was observed in Hoshiarpur district and the least in Barnala district. Hoshiarpur was found mostly to be planted with Kufri Pukhraj and Kufri Chandramukhi cultivars of potato (Table 2). Again the mean aphid population was highest in Hoshiarpur district in both the consecutive years (152.43 and 173.88, respectively). [8] screened a number of potato germplasms against aphid incidence and reported Kufri Badshah, Kufri Chandramukhi and Kufri Pukhraj to be highly susceptible while Kufri Chipsona I and Kufri Chipsona II to be tolerant to aphid infestation. Thus wide plantation of potato with the cultivars mostly susceptible to aphid infestation is the reason behind highest viral disease incidence in Hoshiarpur. Similarly, the mean aphid population in Barnala and Patiala were lowest in both the consecutive years (Figure 3). Again the area under potato cultivation in these districts was found to be maximum with the cultivar Kufri Chipsona and Lady Rosetta (Table 2). Hence the viral disease was lower for both the consecutive years in Barnala and Patiala.

### Serology and symptomatology

In both the year maximum plants were found to be infected with PVX and PVY, exhibiting rugose mosaic and stunting type of symptoms. Plants found infected alone with PVX showed various degree of mosaic and leaf distortion. PVY<sup>n</sup> infected plants showed mosaic and necrosis streaks on leaves and petiole. PVY<sup>o/c</sup> infected plants samples showed mosaic and necrosis type of symptoms. Plants infected with PVS showed blistering, vein deepening and bronzing type of symptoms. Mottling and shiny leaves were found in plants infected with PVA. Leaf rolling at the top portion along with mosaic was observed in plants infected with PVM. Potato plants infected simultaneously with multiple viruses were accompanied with malformation of leaves, rosetting and severe stunting. Among viruses, the highest incidence recorded was of PVX (43.16 %) followed by PVY<sup>n</sup> (34.74 %), PVS (16.84 %), PVM (10.53 %), PVA (9.47%), PVY<sup>o/c</sup> (10.53%), PAMV (5.26 %) and co-infection of PVX- PVY<sup>n</sup> (17.89 %) during 2014 (Figure 2). In the following year of 2015, the highest incidence recorded was of PVX (89.47 %) followed by PVY<sup>n</sup> (75.79 %), PVS (23.16%), PVM (17.89%), PVA (10.53 %), PVY<sup>o/c</sup> (13.68%), PAMV (5.26 %) and co-infection of PVX- PVY<sup>n</sup> (73.68 %) (Figure 2). Reactions of different plant samples against different antisera have been provided in Table 1.

ELISA was conducted over leaf samples collected from seven different localities of Punjab, Pakistan and recorded higher average incidence of PVY (23.1%) as compared to PVX (13.2%) [1]. Survey conducted over Punjab province in Pakistan revealed PVY, PVX, and PVS were the most prevalent followed by PVA, PLRV and PVM, as reported by [7]. All these observations show that PVX and PVY<sup>n</sup> are predominant viruses infecting potato in Punjab, India. Symptoms shown by PVX, PVY<sup>o/c</sup>, PVY<sup>n</sup>, PVS, PVM, PVA

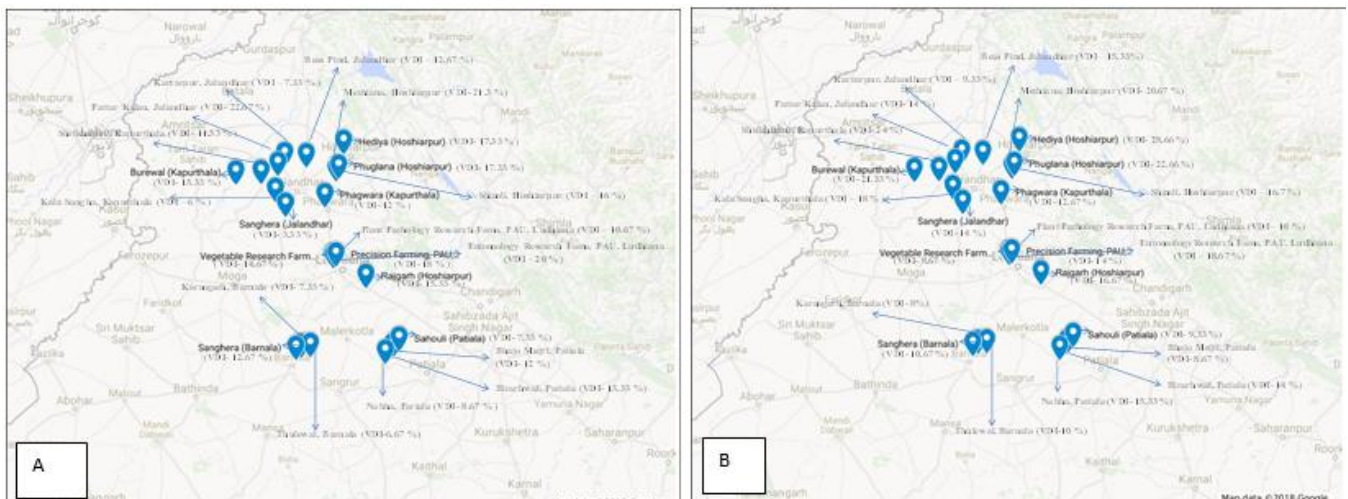
infected potato plants are in line with the findings as cited by [9].

**Aphid population dynamics**

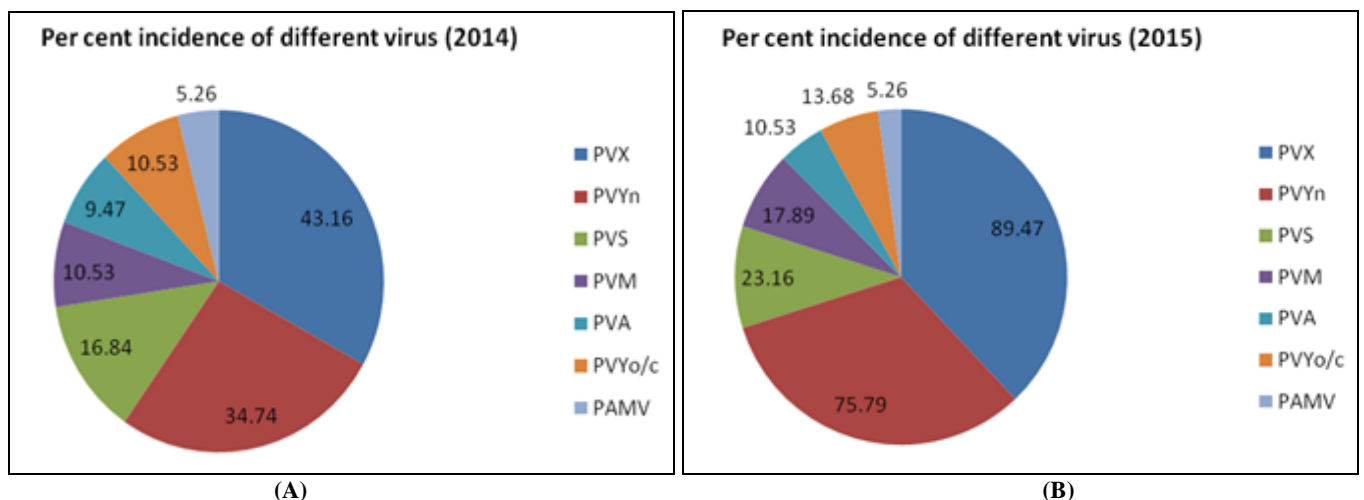
The aphid population started building up from the first week of November and crossed the critical level (20/compound leaves) in Hoshiarpur during the 2<sup>nd</sup> week of December in 2014. Aphid population recorded maximum (380 aphids/100 compound leaves) in Hoshiarpur district during last week of February, 2014. The mean aphid population was maximum in Hoshiarpur (152.43) followed by Ludhiana (141.32), Jalandhar (115.25), Kapurthala (98.37), Patiala (84.75) and Barnala (78.62) during 2014. The population was recorded higher in the following year (2015) where the aphid population crossed the critical level in Hoshiarpur during the first week of December. Similarly, the maximum aphid population was observed in Hoshiarpur (395) during the third week of February in 2015. The mean aphid population recorded highest in Hoshiarpur (173.88) followed by Kapurthala (157.75), Jalandhar (123), Ludhiana (110.63), Patiala (100.19) and Barnala (89.50) during 2015 (Figure 3). There was an increase in the mean aphid population during

the potato growing season by 14.03 from the year 2014 to 2015.

Population of *M. persicae* on the potato crop starts appearing in the third week of December (2-4 aphids/100 compound leaves) and build up to maximum level by end of February in central India as reported by [4]. Aphid population build up by third week of December and reaches the peak by third to fourth week of February in the Gangetic plains of India, as shown by [8]. All these findings support the observation of aphid population dynamics over potato in Punjab. The incidence of PVX, PVY<sup>n</sup>, PVS, PVM and PVY<sup>o/c</sup> was higher in 2015 as compared to 2014. This may be due to increase in mean aphid population by 14.03 during the potato growing season from 2014 to 2015. High mean aphid population in Hoshiarpur district during both the years is the reason behind more viral diseases of potato in Hoshiarpur. Further the viral disease incidence was found to be highly correlated with the aphid population (r=97.49 %) during 2014 and (r=99.59 %) during 2015, which demonstrates the variation in viral disease incidence in different districts on the basis of prevalent aphid population of the district (Fig. 2).



**Fig 1:** (A and B): Viral disease incidence (VDI) obtained from survey (December of 2014 and 2015)



**Fig 2(A and B):** Per cent incidence of different viruses on potato cultivated in Punjab during December 2014 and 2015.

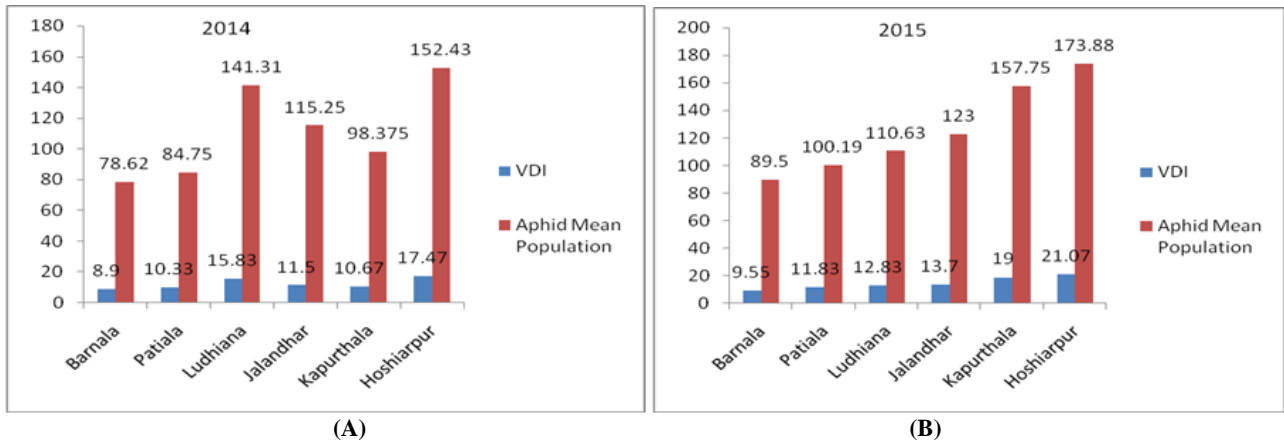


Fig 3(A and B): Aphid population dynamics on potato with relation to viral disease incidence (VDI) in Punjab, India (2014-15 and 2015-16)

Table 1: Reaction of potato plants collected from each six district to ELISA (2014 and 2015)

District	No. of fields	Plant samples infected with different viruses in different districts (in per cent) during 2014 and 2015 (On the basis of ELISA reaction)													
		PVX		PVY <sup>n</sup>		PVY <sup>o/c</sup>		PVS		PVA		PVM		PAMV	
		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Barnala	3	33.3	90.0	26.7	90.0	6.7	10.0	6.7	50.0	6.7	0.0	13.3	10.0	0.0	0.0
Kapurthala	4	33.3	93.3	33.3	40.0	13.3	13.3	13.3	0.0	13.3	26.7	6.7	13.3	0.0	6.7
Hoshiarpur	5	60.0	100.0	53.3	92.9	20.0	14.3	26.7	0.0	20.0	21.4	6.7	21.4	13.3	14.3
Patiala	4	40.0	80.0	0.0	70.0	0.0	40.0	20.0	30.0	0.0	0.0	0.0	30.0	0.0	10.0
Ludhiana	4	50.0	96.7	50.0	70.0	15.0	16.7	25.0	33.3	10.0	13.3	5.0	3.3	10.0	30.0
Jalandhar	4	40.0	68.8	40.0	56.3	6.7	0.0	6.7	6.3	6.7	12.5	33.3	31.3	6.7	0.0

Table 2: Viral disease incidence (VDI) obtained from survey over Punjab, India (December of 2014 and 2015)

District	Village	Variety	VDI %	
			2014	2015
Patiala	Bhojo Majri	Lady Rosetta	12	8.67
	Birarhwal	Lady Rosetta	13.33	14
	Nabha	Kufri Chipsona	8.67	15.33
	Souhali	Kufri Chipsona	7.33	9.33
		<b>Mean</b>	<b>10.33</b>	<b>11.83</b>
Barnala	Karamgarh	Lady Rosetta	7.33	8
	Thulewal	Kuifri Chipsona	6.67	10
	Sanghera	Kuifri Chipsona	12.67	10.67
		<b>Mean</b>	<b>8.89</b>	<b>9.56</b>
Ludhiana	Vegetable Research Farm	Kufri Pukhraj	14.67	8.67
	Plant Pathology Research Farm	Multiple	10.67	10
	Entomology Research Farm	Multiple	20	18.67
	Precision Farming	Multiple	18	14
		<b>Mean</b>	<b>15.83</b>	<b>12.83</b>
Kapurthala	Burewal	Kufri Pukhraj	13.33	21.33
	Kala Sangha	Kufri Pukhraj	6	18
	Tut Kalan	Kufri Pukhraj	11.33	24
	Phagwara	Kufri Pukhraj	12	12.67
		<b>Mean</b>	<b>10.66</b>	<b>19</b>
Jalandhar	Kartarpur	Kufri Badshah	7.33	9.33
	Sanghera	Kufri Chipsona	3.33	14
	Beas Pind	Kufri Badshah	12.67	15.33
	Pattar Kalan	Kufri Jyoti	22.67	14
		<b>Mean</b>	<b>11.5</b>	<b>13.17</b>
Hoshiarpur	Shimli	Kufri Pukhraj	16	16.7
	Methiana	Kufri Pukhraj	21.3	20.67
	Phuglana	Kufri Pukhraj	17.33	22.66
	Rajgarh	Kufri Chandramukhi	15.33	16.67
	Hediya	Kufri Pukhraj	17.33	28.66
	<b>Mean</b>	<b>17.47</b>	<b>21.07</b>	

4. Conclusion

This study showed the per cent distribution of major potato viruses in Punjab, India. The survey of an area involved in

trade of potato crop is mandatory from time to time to keep a check on emergence of new potato viruses or strains of existing viruses.

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