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Survey and Indexing of Weeds Growing around Potato Fields for Their Role as an Inoculum Source for *Potato leafroll virus* (PLRV)

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

A survey and indexing studies was carried out on weeds plants growing around potato fields to identify their role as an inoculum source for *Potato leafroll virus* (PLRV). Symptomatic and asymptomatic foliage samples of 26 weed species were collected and tested by DAS-ELISA and RT-PCR. Out of 783 samples tested, 132 samples showed positive reaction with PLRV antisera. RT-PCR was performed on ELISA positive samples to confirm the results. 110 samples showed amplification of desired band revealing PLRV infection. Weed plants tested positive for PLRV in indexing studies were *Amaranthus viridis* (4), *Chenopodium album* (19), *Datura stramonium* (10), *Physalis minima* (10), *Solanum nigrum* (56) and *Withania somnifera* (12). Results of the study indicated that among the six susceptible weed host plants detected, two weed species (*C. album* and *S. nigrum*) were abundantly growing and acting as important host plants and source of PLRV inoculum in potato growing regions of the present study. These weeds were found growing frequently in vacant fields and wastelands along roadsides and railway tracks near potato growing regions. Colonies of green peach aphids responsible for transmission of virus were observed on host plants infected with virus. Eradication of weed host plants from unused/waste lands bordering potato fields would be helpful in effective management of PLRV infection in potato.

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Keywords: Survey; weeds; potato; aphid; virus.

ABBREVIATIONS

PLRV: Potato leafroll virus; DAS-ELISA: Double Antibody Sandwich Enzyme: linked Immunosorbent Assay; RT-PCR: Reverse Transcription Polymerase Chain Reaction.

1. INTRODUCTION

Potato leafroll virus (PLRV) is one of the most destructive and yield reducing viral pathogen of potato [1]. It is a common viral disease in areas of potato cultivation worldwide and is known to cause yield loss ranging from 10 to 95% [2]. PLRV is transmitted by many viruliferous aphid vectors in a persistent, circulative and non-propagative manner and the most efficient and economically important vector of this virus is the green peach aphid, *Myzus persicae* (Selzer) [3]. *M. persicae* is a small soft bodied polyphagous pest known to survive on 400 plant species of over 50 different plant families [4]. It is the most significant pest of agricultural crops as well as common weed plants [5]. PLRV is transmitted from infected plants to host plants growing at long distance by winged aphids [6]. The virus persists in the mid-gut of the aphid throughout its life cycle and they easily transmit the virus to the host plants by injecting into the phloem.

The important source of PLRV infection is viruliferous aphids and susceptible host plants. Aphid's movement from inoculum sources to healthy plants contributes to the spread of PLRV in cultivated potato crop. They prefer to settle on PLRV-infected plant and then subsequently move to uninfected plants. Important source of PLRV inoculum were susceptible weed plants that are abundant in free and unused lands bordering cultivated crops [7]. Studies have revealed that green peach aphids transmit PLRV more efficiently from solanaceous weed to potato than from potato to potato [8,9]. Increased fecundity of aphids on PLRV infected weed plant was also observed by them which resulted into overcrowding and dispersal of vector to uninfected plants within the cropping area. Solanaceous weed plants are common and abundant in temperate and non-temperate regions of potato cultivation all over the world. Weed plants not only take up nutrients from the soil but also cause harm to potatoes by harboring insects and viral diseases. Studies have proved that weed plants bordering potato fields act as reservoirs for important potato viruses. Many perennial weed species have been identified as infection sources as well as overwintering hosts of different plant viruses [10]. They act as nutrient plants of virus vectors like aphids, thrips etc and play important role in virus ecology and epidemiology.

A relatively wide host range among plant species provides opportunities for widespread distribution and perpetuation of plant viruses in different host plants from season to season. Many common weed species have been identified as hosts for PLRV [7]. PLRV has been detected in weed plants like *Amaranthus retroflexus* [11], *Capsella bursa-pastoris* [10,12], *Chenopodium album* [10], *Datura stramonium* [11,13,14], *Solanum nigrum* [12], *Solanum sarrachoides* [8,9,15], and *Solanum viarum* [16]. Survey and indexing of weed species showing symptoms of PLRV by serological and molecular methods may be valuable in controlling the spread of PLRV in potato growing regions by eradicating host plant species.

Potato cultivation occupies nearly 50% of the total area of vegetable crops in Punjab and it is one of the largest potato producing states in India [17]. Weed species growing within potato ecosystem is a challenge for potato growers and is known to be a major component limiting potato production in Punjab [17]. They host many important pests and diseases. Weeds plants known to be host for PLRV were found growing in vegetable producing regions of the present study. The present work was carried out with an objective to index weed plants as host and reservoir for PLRV, a most important and damaging viral disease commonly found in potato growing regions of the world. Many different weed species have been identified as host for PLRV in literature [7,11]. Role of weeds that are commonly found on vacant land near potato fields as alternative virus hosts has not been reported. Survey and indexing of weeds growing in the regions of present study was carried out to provide information about weed plants responsible for PLRV infection. Indexing studies of weed species will allow identification of PLRV inoculum sources and will, in turn, be useful for formulation of virus and weed management strategies.

2. MATERIALS AND METHODS

2.1 Survey and Sample Collection

Survey was conducted during September 2014 to March 2016 on vacant or unused land along roadsides, railway line tracks and sides of canals near potato fields infected with PLRV in Amritsar (Latitude 31°38'N, Longitude 74°51'E) and Jalandhar (Latitude 31.326°, Longitude 75.57°) regions of the Punjab. The locations selected for sampling has loamy soils. The maximum and minimum temperature during potato growth was 18.1-32℃ and 1.1-18.0℃, respectively. The potato varieties grown by farmers in these two regions were *Kufri Pukhraj*, *K. Chipsona-1*, *K. Chandramukhi*, *K. Jyoti*, *K. Bahar*, *K. Lauvkar*, *K. Lalima* and *K. Ashoka*. Experimental work was carried out at Plant Biotechnology Laboratory, Khalsa College Amritsar (India). Weed species that exhibited visible symptom or were without any conspicuous symptoms of viral diseases were selected for sample collection and surveyed regularly during growth and harvesting of potato (Table 1). Foliage samples of fully grown and mature weed plants were collected during season-long survey and transported to lab for indexing of virus. The same areas of potato cultivation were regularly sampled at two-four week intervals in both years of this study and schedule of survey was planned to ensure that weed species suspected to be host of vector and virus would be collected during sampling. Samples were collected from 26 weed plants of 17 different families. The purpose of this study is to index weed species growing in potato ecosystem and determine their role in spread of PLRV in potato crops. Such research is necessary for the development of strategies for virus control.

2.2 Virus Indexing

PLRV infections in foliage samples of weed plants were assayed by DAS ELISA and RT-PCR. The detailed method as described by Clark and Adams [18] was followed for indexing by DAS-ELISA. Anti PLRV IgGs, enzyme conjugate, positive and negative controls of Agdia, Inc (Elkhart, Indiana) were purchased and used for indexing of weed samples by DAS-ELISA. DAS-ELISA was performed on sap extracted from leaves of weeds. Absorbance values were read at 405 nm on Multiskan EX plate reader (Thermo Fisher Scientific, Vantaa, Finland). The reaction was considered positive only when the mean absorbance value was more than two times of negative control.

RT-PCR was performed on leaf samples of weed plants collected and transported to laboratory in liquid nitrogen. RNA was extracted from frozen leaf samples using TRIzol reagent (Invitrogen, USA). Reverse transcription (RT) reaction was carried out in a total reaction volume of 50 µl in 0.2 ml thin walled tube using 200 units of M-MLV reverse transcriptase (G-Biosciences, India), 200 ng down stream primer, 10 mM of dNTPs mix, 25 Units of RNase inhibitor, 10 µl total RNA and 5X reverse transcriptase buffer. RT reaction was carried out at 37℃ for 1.15 h followed by incubation at 70℃ for 5 min and then immediate transfer to ice. The upstream (5'CGCGCTAACAGAGTTCAGCC3') and downstream (5'GCAATGGGGGTCCAACTC AT3') primers for PLRV were used to yield amplification of 336 base pairs in RT-PCR assay [19]. The detailed procedure used for indexing of samples by DAS-ELISA and RT-PCR was described in previous report [1]. PLRV infected and PLRV-free plants confirmed by indexing and sequence validation in previous studies [20,21] were used as internal controls in RT-PCR experiments. Amplification obtained in RT-PCR of weed samples was sequenced and BLAST analyzed for confirmation of the indexing results.

3. RESULTS AND DISCUSSION

3.1 Survey of Weed Species

Incidence of PLRV disease has caused serious loss to potato growers, particularly in recent years. Potato is grown two to three times in a year under diverse agro-climatic conditions. In last few years high incidence of PLRV has been reported and efforts were made to provide virus free plants using *in vitro* techniques [1,20,21]. Many weed plants known to host for virus and its vector were found growing in potato ecosystem. Use of virus-free planting material alone would not help in effective management of PLRV unless weeds serving as infection source of PLRV were not eradicated. Control of weeds within fields is a routine process followed by potato growers in Punjab [17]. However during survey of potato fields, it was observed that the vacant or unused land along roadsides and railway track near potato growing regions were heavily infested with diverse type of weed flora. Foliage samples of weed plants growing on unnoticed areas nearby potato fields were collected and transported to laboratory with an aim to identify the source of inoculum for PLRV in weed flora. PLRV is easily transmitted by green peach aphids from infected to uninfected

plants. Colonies of green peach aphids were observed on lower side of leaves in some of the *Chenopodium album, Solanum nigrim* and *Datura stramonium* plants. These aphids were more common on weeds during harvesting season of potatoes. The research on virus-infected weeds growing in region where potato crop is cultivated has not been carried out earlier. Many studies have proved outbreak of virus in a cultivated crop has been associated with the occurrence of the same viruses in weeds [7,14, 16,22-24]. Areas of potato cultivation with high incidence of PLRV and heavy infestation of weeds on wastelands adjacent to fields were selected, surveyed and sampled. Twenty six weed species of seventeen different families (Table 1) were found growing on unused land adjacent to potato fields like sides of canals, along roadsides and railway line tracks. In order to identify source of inoculums all these weeds species growing in close proximity to potato fields infected with PLRV were only sampled. Some of the weed plants like *Chenopodium album*, *Datura stramonium*, *Physalis minima*, *Solanum nigrum* showed typical virus like symptoms (Table 1). Symptoms alone were not used as criteria for collection of foliage samples from weeds as PLRV infection does not show symptoms on all the host plants. Asymptomatic weeds growing near infected potato fields were also sampled. Sometimes symptoms on host plants may be confused with those of nutritional deficiency, drought, water-logging or herbicide injury. Knowing weed hosts of PLRV in potato growing region could be the basis for PLRV control by eradicating them from unnoticed areas near potato fields. Earlier, Smith et al. [7] have determined the role of weed species known to be host for viruses in season-long survey of common weeds growing near onion and potato fields. Knowledge about weed species known to be host of viruses in cultivated crops would be help in understanding the epidemiology of these viruses in cropping systems.

3.2 Indexing of Weed Species

A total of 1226 samples (with/without virus symptoms) of 26 different weed plants were collected during survey from September 2014 to March 2016 and 783 samples were indexed by DAS-ELISA. 132 samples showed positive reaction to PLRV antisera. Among the weed species sampled and tested, *Amaranthus viridis* (5), *Chenopodium album* (21), *Datura stramonium* (13), *Physalis minima* (12), *Solanum nigrum* (62) and *Withania somnifera* (17) were found positive for PLRV in DAS-ELISA (Table 1). Only two samples of *Malva neglecta* were found positive for virus in DAS-ELISA. Other than these seven plant species, none of the other weed samples were detected positive for virus in serological indexing. RT-PCR was performed only on 132 ELISA positive samples to further confirm the results. Out of them, 110 samples belonging to *Amaranthus viridis* (4), *Chenopodium album* (19), *Datura stramonium* (10), *Physalis minima* (10), *Solanum nigrum* (56) and *Withania somnifera* (12) produced a band of 336 bp revealing PLRV infection (Fig. 1). The sequence obtained from RT-PCR products of weed samples showed homology in BLAST analysis with portion of viral coat protein gene. PLRV was confirmed in *Amaranthus viridis, Chenopodium album, Physalis minima, Solanum nigrum, Datura stramonium* and *Withania somnifera* plants growing near infected potato fields. The plants of *Solanum nigrum, Datura stramonium* and *Chenopodium album* growing on wasteland around potato fields were found infected and they serve as natural reservoirs of PLRV in vegetable producing areas of Punjab.

Two samples of *Malva neglecta* detected positive in ELISA does not yielded any amplification in RT-PCR and were noted to be negative for virus infection (Fig. 1). Solanaceous weeds in potato growing region of Punjab are very common along roadsides near potato fields and they frequently go unnoticed and act as important source of PLRV inoculum for next season crop. In present study, RT-PCR indexing of samples showed that PLRV infection was mainly present in solanaceous weeds like *Datura stramonium, Solanum nigrum, Physalis minima* and *Withania somnifera.* Previous investigations have also proved the role of solanaceous wild plants in spread of PLRV [14,25]. Among non-solanaceous plants *Chenopodium album* is found to be important host plants and source of PLRV inoculum. It was observed that *S. nigrum* and *C. album* are potential PLRV infection sources to the aphids visiting potato fields. These two weed species were frequently present on wastelands along roadsides and railway track near fields commonly used for cultivating potatoes in previous years. Survival of weed host plants on such areas after harvesting of potato presents a continuum of host availability for virus and aphids. Infected weeds potentially affect population build-up of aphids and contribute towards spread of PLRV disease [9]. Weeds persist there after growing season, remain unnoticed and are not eradicated by potato

growers. Information about these weeds as inoculums sources will be useful for both potato growers and plant protection agencies involved in seed potato production.

Important sources of PLRV inoculums for newly cultivated disease free potato plants would potentially include aphids surviving on weed plants that are abundant in free or unused lands near potato fields along roadsides. Colonies of green peach aphids were observed on lower surface of leaves of host plants. Aphid movement easily transmit virus from reservoir to cultivated plants and contributes to virus spread in a crop field. Weed host plants detected positive in virus indexing and green peach aphids colonizing on them were playing important role in spread of PLRV in the areas sampled and studied. Previous studies have proved that green peach aphid prefer to settle on virus infected weed plant before moving to cultivated crop and transmit PLRV more efficiently from weed to potato than from potato to potato [8,25]. Many other studies have proved that weed plants growing nearby cultivated fields serve as hosts and reservoir for plant viruses and vectors and they transmit viral diseases to cultivated crops [7,12,14,23,25]. Aphids acquire PLRV from infected weeds concentrated along roadsides; railway tracks etc. and then transmit the virus to susceptible crops. The presence of susceptible weeds plays an important role in PLRV epidemiology by increasing aphid populations and PLRV incidence in potato fields [24].

Identification of alternate hosts or weeds responsible for spread of plant viral diseases and their eradication would be helpful in effective management of viral pathogens [23]. These weeds while growing along the edges of potato fields act as important reservoirs for both virus and vector and play significant role in dissemination of viral diseases in potato ecosystem [24,26]. It was observed that *Solanum nigrum* plants tested positive in DAS-ELISA and RT-PCR have heavy infestation of aphids. These plants might be serving as reproductive host for the virus vector and reservoir for virus infection in region of the present study. Presence of weed hosts around potato field significantly hastens viral spread in an agro-ecosystem as they allow carry-over of viruliferous aphids and virus inoculum [14]. The spread of PLRV among susceptible weeds by aphids before the potato crop is available for infection creates a situation highly conducive for spread of PLRV to newly planted susceptible crops. Insecticidal management of aphid vectors may not work to reduce the incidence of PLRV unless measures were taken to identify and remove virus inoculum sources within and outside the potato ecosystem. Considering the importance of *S. nigrum* in spread of PLRV infection, their eradication before planting next season crop should be given high priority. Studies related to role of weeds in spread of plant viruses transmitted by insects vectors have indicated that removal of weed host would be as effective as chemical control of aphids [23]. Potato growers using certified seed potatoes were unable to control PLRV spread due to presence of infected weeds as potential infection sources around the fields. Awareness of potato growers about possible role of weed hosts growing nearby fields would prove helpful in efficiently controlling PLRV in the region of the present study.

Fig. 1. Agarose gel electrophoresis of RT-PCR products of weed samples showing amplification of desired (336 bp) coat protein gene fragment of PLRV (Lanes 1: Negative control, Lane 2: *Amaranthus viridis,* Lane 3: *Chenopodium album,* Lane 4-5*:Malva neglecta*, Lane 6: *Withania somnifera,* Lane 7: 100 bp DNA ladder, Lane 8: *Datura stramonium,* Lane 9-10: *Physalis minima,* Lane 11: *Solanum nigrum* and Lane 12: Positive control)

Table 1. Weed species growing in or adjacent to potato fields indexed for PLRV infection by ELISA and RT-PCR

Weed Species	Family	Common name (Local name)	Symptoms observed	No of plants sampled	Number of plants tested*	
					DAS-ELISA	RT-PCR
Achyranthes aspara	*Amaranthaceae*	Chaff flower (Puth kanda)	Chlorosis	47	23	NP
Amaranthus viridis	*Amaranthaceae*	Pigweed (Cholai)	No symptoms	30	30 (5)	5 (4)
Anagallis arvensis	*Primulaceae*	Poison weed (Billi booti)	No symptoms	45	25	NP
Argemone mexicana	*Papaveraceae*	Prickly Poppy (Sial-kanta)	No symptoms	26	20	NP
Ageratum conyzoides	*Asteraceae*	Goat weed (Jangli pudina)	Leaf distortion	72	20	NP
Boerhavia diffusa	*Nyctaginaceae*	Pigweed (Itsit)	Chlorosis	19	15	NP
Calotropis procera	*Asclepiadaceae*	Gaint weed (Aak)	No symptoms	22	15	NP
Cannabis sativa	*Cannabinaceae*	Neck weed (Bhang)	Mosaic	98	42	NP
Circium arvense	*Asteraceae*	Creeping thistle (Kandai)	No symptoms	12	12	NP
Coronopus didymus	*Brassicaceae*	Swine-cress (Jangli halon)	Chlorosis of leaves	95	36	NP
Chenopodium album	*Chenopodiaceae*	Goosefoot (Bathu)	Chlorotic and Necrotic spots	91	91 (21)	21 (19)
Convolvulus arvensis	*Convolvulaceae*	Field bindweed (Hiran khuri)	No symptoms	24	20	NP
Datura stramonium	*Solanaceae*	Jimson weed (Sada Datura)	Interveinal chlorosis	20	20 (13)	13 (10)
Lantana camara	*Verbenaceae*	Lantana (Raimuniya)	No symptoms	9	6	NP
Malva neglecta	*Malvaceae*	Mallow (Sonchala)	Leaf yellowing	65	50 (2)	2 (0)
Medicago denticulata	*Fabaceae*	Burclover (Maina)	Chlorosis	59	27	NP
Parthenium hysterophorus	*Asteraceae*	Congress grass (Gajar booti)	No symptoms	87	49	NP
Physalis minima	*Solanaceae*	Ground Cherry (Rasbhari)	Mosaic	21	21(12)	12 (10)
Phyllanthus urinaria	*Euphorbiaceae*	Gripeweed (Bhuiamla)	Chlorosis	20	9	NP
Ricinus communis	*Euphorbiaceae*	Castor (Erand)	Chlorosis	31	13	NP
Rumex dentatus	*Polygonaceae*	Toothed dock (Jangli palak)	Chlorotic and Necrotic spots	65	27	NP
Solanum nigrum	*Solanaceae*	Black nightshade (Makko)	Leaf distortion, Mosaic	117	117 (62)	62 (56)
Sonchus oleraceus	*Asteraceae*	Sow thistle (Dodhak)	Chlorosis	84	35	NP
Vicia sativa	*Fabaceae*	Common vetch (Akri)	No symptoms	16	11	NP
Withania somnifera	*Solanaceae*	Winter cherry (Asgandh)	Chlorotic spots	40	40 (17)	17 (11)
Xanthium stromarium	*Asteraceae*	Cocklebur (Chota Gokhuru)	Chlorosis of leaves	11	9	NP
			Total sampled/tested	1226	783	132

**In parentheses, number of plants detected positive by DAS-ELISA and RT-PCR, NP = Not performed*

4. CONCLUSION

PLRV is one of the most important virus infecting potato crops. Survey and indexing studies concludes that some of the weed plants growing next to or nearby potato fields have role in PLRV spread. Virus is rapidly spread by viruliferous aphids immigrating from infected weed plants like *Chenopodium album, Datura stramonium, Physalis minima, Solanum nigrun* and *Withania somnifera* growing nearby potato fields. These weed plants are susceptible to PLRV and grows abundantly on unused areas bordering potato fields. Removal of susceptible weeds can reduce the incidence of PLRV on potatoes.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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