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Species Composition and Distribution of the Vector Aphids of PVY and PLRV in India

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Abstract

Aphid transmitted viruses are the most significant constraint for quality seed potato production. *Potato virus Y* and *Potato leaf roll virus* are the most important aphid transmitted viruses in potato, which can be transmitted by a large number of aphid species. In order to determine the species composition of aphids infesting potato crops and to determine their viruliferous nature, aphid samples were collected from all the potatoes growing zones in India. Fourteen distinct aphid species were identified from 541 samples using morphological and molecular characterization. The identified species include *Aphis craccivora*, *A. gossypii*, *A. fabae*, *A. spiraecola*, *Aulacorthum solani*, *Macrosiphum euphorbiae*, *Rhopalosiphum rufiabdominale*, and *Myzus persicae* among the colonizing species and *A. nerii*, *R. padi*, *R. maidis*, *Hyadaphis coriandri*, *Brevicoryne brassicae*, and *Lipaphis erysimi* among the non-colonizing species. *M. persicae* and *A. gossypii* were the most abundant and most widespread species on potato in the country. Based on whole body testing of aphid samples through RT-PCR, *M. persicae*, *A. gossypii*, *A. solani*, and *M. euphorbiae* were found positive for PVY while *M. persicae* and *A. gossypii* tested positive for PLRV, which could potentially transmit the respective viruses in potato crops. The current study revealed that aphid species other than *M. persicae* could be important in determining the virus incidence in seed crops in the country. Therefore, management decisions should include such information to maintain the health standard of seed stocks.

Keywords Aphid vectors · Potato virus · Seed potato · Viruliferous · Virus transmission

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Introduction

Aphids and aphid transmitted viruses are the most important challenge for the production of quality seed potato throughout the world. The viruses transmitted by aphids result in considerable yield reductions and progressive degeneration of seed stocks (Radcliffe and Ragsdale 2002). More than thirteen potato viruses are transmitted by aphids of which *Potato virus Y* (PVY) and *Potato leaf roll virus* (PLRV) are the most important (Brunt 2001; Radcliffe and Ragsdale 2002).

Globally, around 65 species or species groups of aphids have been demonstrated to transmit PVY in a non-persistent manner (Lacomme et al. 2017), and 13 species are capable of transmitting PLRV through a persistent manner in potato crops (Khaled et al. 2018). Although only around 25 species of aphids are known to colonize potato crops, more than 110 species of aphids are known to visit potato plants transiently (Blackman and Eastop 2000; Steinger et al. 2015; Shah et al. 2018). The non-colonizing aphids become important sources for spreading non-persistent viruses like PVY while the persistent viruses like PLRV are mostly transmitted by colonizing species of aphids (Boquel et al. 2011).

In India, PVY, PLRV, *Potato virus A* (PVA), *Potato virus S* (PVS), and *Potato virus M* (PVM) are the most important potato viruses vectored by aphids (Awasthi and Verma 2017; Raigond et al. 2020). The vector-borne viruses cause up to 20–50% yield loss in potato crops (Tiwari et al. 2012). Among these, about 80 percent of the seed degeneration is attributed to PVY and PLRV (Pushkarnath 1967) in the country.

A large number of aphid species are reported on potato crops from different parts of India. The most frequently reported species are *Myzus persicae* Sulzer, *M. ornatus* Laing, *Aphis gossypii* Glover, *A. craccivora* Koch, *A. fabae* Scopoli, *A. medicaginis* Koch, *A. rhamnii* (= *nasturtii*) Kaltenbach, *A. spiraecola* Patch, *Macrosiphum euphorbiae* (Thomas), *M. rosae* (Linn.), *Rhopalosiphum maidis* Fitch, *R. rufiabdominalis* (= *rufiabdominale*) Sasaki, *R. nymphaeae* (Linn.), *Tetraneura nigriabdominalis* (Sasaki), *Rhopalosiphoninus latysiphon* David, *Lipaphis erysimi* Kaltenbach, and *Brevicoryne brassicae* (Linn.) (Pushkarnath 1959, Bindra and Sekhon 1971; Verma 1977; Sekhon and Bindra 1979; Kashyap and Verma 1982; Misra and Agrawal 1987; Kumara et al. 2017). Although most of these are potential vectors of different potato viruses, their role and importance as vectors are yet to be elucidated under Indian conditions.

For the production of quality seed, the potato crops are cultivated during a period of low aphid activity in India, the 'seed plot technique'. The window is based on the population growth of *M. persicae* in the sub-tropical plains of India. It is recommended to cut the haulms as soon as the population of *M. persicae* crosses the threshold limit of 20 aphids per 100 compound leaves (Pushkarnath 1959, 1967). As *M. persicae* is the most efficient vector of PVY and PLRV, the management decisions based on its population size served the purpose of quality seed potato production well by keeping the virus incidence under check. Of late, there is a large-scale spread and incidence of PVY strains throughout the country (Hegde et al. 2020). Since the non-colonizing aphids mainly spread PVY, the current management strategy may not provide a satisfactory level of control of its incidence. In addition, the number of aphid species infesting potato crops reported has considerably increased since the inception of seed plot technique. Therefore, it has become imperative to consider the population size and flight activity of other vector aphid species as well while taking management decisions.

With this background, present study was taken up to identify the common aphids infesting potato crops in India to develop a species profile and preliminary distribution maps and to determine their viruliferous nature with respect to PVY and PLRV.

Materials and Methods

Aphid Sampling

Aphid samples (*N*=541) were collected from all potato growing zones in the country including the Indo-Gangetic plains, North-eastern hills, North-western hills, the plateau and the southern hills from 2014 to 2016. The sampling sites were spread in thirty districts of 17 different states (Fig. 1). Since potato is cultivated in different seasons in different agro-ecologies in the country, aphids were sampled at different times at different locations, e.g. during *Rabi* season in the Indo-Gangetic plains (October–January), during *Kharif* (June–September) in the plateau region, and during the summer season in the hills (April–August).

Roving surveys were conducted at multiple locations in the target districts for collection of aphid samples from potato crops during multiple growing seasons. Individuals from both the colonizing and non-colonizing species were collected directly from the foliage of plants. In each sampling bout, aphids were collected from 30 plants in each field, from three leaves selected from upper, middle, and lower strata in each

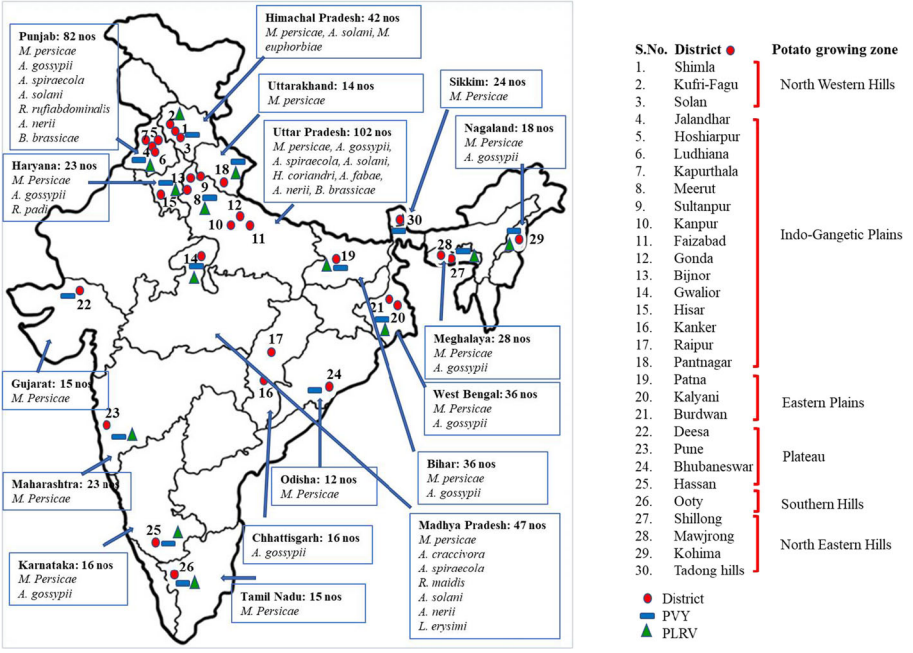

Fig. 1 Graphical representation of sampling sites and distribution of aphid species, PVY and PLRV on potato in India. Numbers following the name of the state indicate sample size. The red dots on the map with numbers represent the sampled districts. Lists of aphids in blue boxes represent distribution of aphid species in the respective states. Blue rectangle represents distribution of PVY. Green triangle represents distribution of PLRV.

plant (Hafez 1961). Samples were collected on two occasions (early and mid) in each growing season. The aphid samples were preserved in 99% ethanol in cryo vials (5 ml), labelled for location and date of sampling and brought to laboratory. The aphid samples were sorted for possible morphospecies and counted. Selected specimens were processed and mounted on microscope slides following the standard protocols described by Foottit and Maw (2000).

Identification

Identification based on morphological characteristics (like antennae, scelerites on abdominal tergites, siphunculi, and cauda) was undertaken using standard taxonomic keys (Blackman and Eastop, 2000). Besides, specimens were sent to ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India, the nodal institute for aphid identification. Reference samples were kept in the Entomological Laboratory of Division of Plant Protection, ICAR-Central Potato Research Institute (CPRI), Shimla (India).

For molecular characterization, representative samples from each morphospecies (ca. 10% of the samples) were used, while as for virus detection, all the remaining specimens were used. Total nucleic acid (DNA and RNA) was isolated from individual aphid samples using the print capture technique (Nagata et al. 2004; Gawande et al. 2007, 2011; Sridhar et al. 2016; Raigond et al. 2020). The eluted nucleic acids were used for species identification and determination of the viruliferous nature of the aphid samples. Mitochondrial DNA was amplified using COI primers for aphid species identification, and RNA was reverted to cDNA using a cDNA synthesis kit (Fermentas Life Sciences, Germany). Then, cDNA was used as usual for RNA virus detection.

For species identification, we amplified the mt*COI* gene using the primer set Sense, LCO-1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′, and Antisense, HCO-2198, 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Hebert et al. 2003). PCR was performed in a thermal cycler (Applied Biosystems 9700) using the cycling parameters consisting of an initial denaturation at 94°C for 4 min followed by 35 cycles at 94°C for 30 s, annealing at 50°C for 45 s, an initial extension step at 72°C for 1 min, and a final extension at 72°C for 20 min (Rebijith et al. 2013). The total reaction volume of 25 μl having 1.0 ul of each primer (10 mM), 2.5 ul of 10× taq buffer, 2.5 ul of 2.0 mM dNTP, 1.0 ul of 2.5 mM $MgCl_2$, and 1.0 ul of 0.5 unit Taq DNA polymerase including 4 μl template DNA in 12 ul sterile distilled water. The amplified products were resolved on 1.0% agarose gel, stained with ethidium bromide (10 μg/ml) and visualized in a gel documentation system. An amplicon of 658 bp of mtCOI gene was observed on gel (Fig. 2). The sharp bands were gel eluted using Qiagen gel elution kit

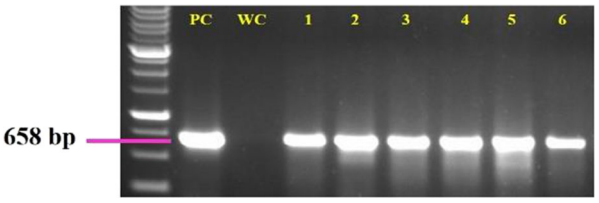

Fig. 2 Amplification of mitochondrial COI gene of aphid species. Marker 1kb ladder, PC positive control, WC water/negative control, lane 1–6 aphid samples of Jalandhar and Modipuram

(Germany) by following user protocol. The purified eluted product was further used for gene sequencing. The purified mt DNA was sequenced by employing 3500 Genetic Analyser, Applied Biosystems (Hitachi). DNA sequences were aligned using ClustalW programme implemented in MEGA ver. 6.0. Software (Tamura et al. 2013). A homology search was done using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/), and all the sequences generated were deposited in NCBI-GenBank (Table 1). We analysed 60 different sequences of aphid species with other closely related available sequences from NCBI GenBank, and fourteen species of aphids were identified. These sequences were used for phylogenetic analysis using MEGA version 6.0. Pairwise genetic distance between aphid and their related species was obtained based on Kimura's two parameter model and used for estimating intraspecific or interspecific genetic distances (K2P) across species (Letunic and Bork 2021). A phylogenetic tree of aphids and their related species was constructed using the K2P with boot strapping of 1000 times as implemented in MEGA version 6.0.

Determination of the Viruliferous Nature of Aphids

Using the extracted nucleic acids as described above, aphid samples were processed for their viruliferous nature with respect to PVY^{O} and PLRV through RT-PCR. The total extracted RNA was quantified using Nano drop (Thermo Scientific, Leon-Rot, Germany), and cDNA was synthesized using random primers with Revert Aid c-DNA synthesis kit. The reverse transcription mixture, i.e. 4.0 μl of 5× buffer, 2.0 μl of 10 mM each dNTP mix, 1.0 μl of 20 U/ml RNase inhibitor, 1.0 μl of 0.2 mg/ml random primer, 1.0 μl of 200 U/μl RT enzyme, 6.0 μl of template RNA, and 5.0 μl of RNase free water, was added to provide a final volume of 20 μl. All the reactions were carried out in ice chilled condition to avoid premature cDNA formation and to minimize the risk of RNA degradation. The reaction mixture was incubated at 25°C for 05 min, 42°C for 59 min, and 75°C for 10 min. A desired concentration of 20 ng μl^{-1} of cDNA was used for PCR amplification. Polymerase chain reaction was carried out using coat protein specific primers for PVY^{o} (forward PVY-FCP-5′-ACGT GGTATGAGGCAGTGCGGA-3′ and reverse PVY-FCP-5′-ATGTGCGCTTCCCT AGCCCTCA-3′) and PLRV (forward PLRV-FCP-5′-CTAACAGAGTTCAG CCAGTGGTTA-3′ and reverse PLRV-RCP-5′-CGGTATCTGAAGATTTTCCA TTTC-3′) (Venkateswarlu et al. 2016; Raigond et al. 2014; Sridhar et al. 2021a). Duplex PCR reaction for PVY and PLRV was carried out at 62°C in a thin walled 0.2 ml PCR tubes (Gene Amp PCR 9700, Applied Biosystems, USA) with a reaction mixture of 20 μl containing 2.5 μl of 10× Taq buffer A, 1.5 μl of 2 mM dNTP mix, 1.0 μl of 10 pM forward and reverse primers each, 2 μl of cDNA template, 1.0 μl of 1.0 U/μl of Taq DNA polymerase, and 11 μl sterile nano pure water. The PCR conditions used to amplify coat protein genes were as follows: initial denaturation at 94°C for 2 min, 94°C for 30 s, and annealing at 62°C for 45 s, followed by extension at 72°C for 35 cycles for 1 min along with final extension step at 72°C for 5 min (Venkateswarlu et al. 2016). The amplified products were resolved in 1.0% agarose gel and visualized in gel documentation system. The bands of the amplified coat protein genes indicated the presence of virus in the aphid sample.

Table 1 Details of aphid species collected on potato from different parts of India

Species	Location	Latitude, longitude	Voucher specimen	GenBank accession number/s
Aphis craccivora	Gwalior, Madhya Pradesh	26.2183° N, 78.1828° E	Gwalior A4	KY613935
Aphis fabae	Manikpur (Chitrakoot), Uttar Pradesh	25.1094° N, 81.0755° E	Manikpur A14	KY613936
Aphis gossypii	Hisar, Haryana	29.1492° N, 75.7217° E	Hisar_A88 Hisar_A86	KY586070 KY586071
	Modipuram (Meerut), Uttar Pradesh	28.9845° N, 77.7064° E	Modipuram_CA1 Modipuram_CA2 Modipuram_CA3 Modipuram_BA2 Modipuram_A32 Modipuram_BA3	KY586072 KY586073 KY586074 KY586075 KY586076 KY586077
Aphis nerii	Jalandhar, Punjab	31.3260° N, 75.5762° E	Jalandhar A1	KY606281
	Modipuram (Meerut), Uttar Pradesh	28.9845° N, 77.7064° E	Modipuram A43	KY606282
Aphis spiraecola	Gwalior, Madhya Pradesh	26.2183° N, 78.1828° E	Gwalior A3 Gwalior A5 Gwalior A6 Gwalior A7 Gwalior A8	KY586078 KY586079 KY586080 KY586081 KY586082
	Jalandhar, Punjab	31.3260° N, 75.5762° E	Jalandhar A2 Jalandhar A3	KY586083 KY586084
	Modipuram (Meerut), Uttar Pradesh	28.9845° N, 77.7064° E	Modipuram A43.1	KY586085
Aulacorthum solani	Modipuram (Meerut), Uttar Pradesh	28.9845° N, 77.7064° E	Modipuram CA4	KY606275
	Gwalior, Madhya Pradesh	26.2183° N, 78.1828° E	Gwalior A59	KY606276
	Shimla, Himachal Pradesh	31.1048° N, 77.1734° E	Sml A61	KY606277
	Jalandhar, Punjab	31.3260° N, 75.5762° E	Jalandhar A3 Jalandhar A3.1 Jalandhar A4	KY606278 KY606279 KY606280
Brevicoryne brassicae	Bijnor, Uttar Pradesh	29.3721° N, 78.3842° E	Bijnor A49	KY586091
	Jalandhar, Punjab	31.3260° N, 75.5762° E	Jalandhar A49	KY586086

Table 1 (continued)

Species	Location	Latitude, longitude	Voucher specimen	GenBank accession number/s
	Kalyani, West Bengal	22.9751° N, 88.4345° E	Kalyani A10	KY586087
	Shimla, Himachal Pradesh	31.1048° N, 77.1734° E	Sml A70 Sml A69 Sml A71	KY586088 KY586089 KY586090
Lipaphis erysimi	Kankar, West Bengal	23.5489° N, 86.8023° E	Kankar A1	KY613937
Hyadaphis coriandri	Modipuram (Meerut), Uttar Pradesh	28.9845° N, 77.7064° E	Modipuram A41 Modipuram A45	KY613933 KY613934
Macrosiphum euphorbiae	Shimla, Himachal Pradesh	31.1048° N, 77.1734° E	Shimla A7	KY613938
Myzus persicae	Hisar, Haryana	29.1492° N, 75.7217° E	Hisar_A76 Hisar_A78	KY586050 KY586051
	Deesa, Gujarat	24.2324° N, 72.1991° E	Deesa_A50 Deesa_A51 Deesa_A53 Deesa_A54	KY586052 KY586053 KY586054 KY586055
	Modipuram (Meerut), Uttar Pradesh	28.9845° N, 77.7064° E	Modi_MP1 Modi_MP2 Modi_MP3 Modi_MP4 Modi_MP5	KY586056 KY586057 KY586058 KY586059 KY586060
	Patna, Bihar	25. 3532° N, 85.429° E	Patna_A56 Patna_A58	KY586061 KY586062
	Kalyani, West Bengal	22.9751° N, 88.4345° E	Kal_A5 Kal_A6 Kal_A7 Kal_A8 Kal_A9 Kal_A10	KY586063 KY586064 KY586065 KY586066 KY586067 KY586068
	Pune, Maharashtra	18.4737° N, 73.7558° E	Pune_A12	KY586069
Rhopalosiphum padi	Hisar, Haryana	29.1492° N, 75.7217° E	Hisar A80	KY613940
Rhopalosiphum rufiabdominalis	Patna, Bihar	25.5934° N, 85.0761° E	Patna A57	KY613941
	Jalandhar, Punjab	31.3260° N, 75.5762° E	Jalandhar A6	KY613942
Rhopalosiphum maidis	Gwalior, Madhya Pradesh	26.2183° N, 78.1828° E	Gwalior A2	KY613939

Results

A total of 541 aphid samples were processed for species identification and distribution across the agro-ecologies in India. In all, fourteen species of aphids belonging to eight distinct genera were identified (Table 1). The identified species were green peach aphid, *Myzus persicae* (Sulzer); cotton aphid, *Aphis gossypii* Glover; potato aphid, *Macrosiphum euphorbiae* (Thomas); cabbage aphid, *Brevicoryne brassicae* Linnaeus; foxglove aphid, *Aulacorthum solani* (Kaltenbach); mustard aphid, *Lipaphis erysimi*; black bean aphid, *Aphis fabae* group Scopoli; coriander aphid, *Hyadaphis coriandri* (Das); rice root aphid, *Rhopalosiphum rufiabdominale* (Sasaki); spiraea aphid, *Aphis spiraecola*; oleander aphid, *Aphis nerii* Boyer de Fonscolombe; groundnut aphid, *Aphis craccivora* Koch; grain aphid, *Rhopalosiphum padi* (L.); and maize aphid, *Rhopalosiphum maidis* (Fitch).

The mt*COI* nucleotide sequences of the identified species were deposited in the National Centre for Biotechnology Information (NCBI) database. The sequences showed 98–100% similarity with reported sequences in the NCBI database. No pseudogenes were amplified as indicated by the absence of stop codons within the sequences and similarity of base composition, with no indels.

The green peach aphid was the most commonly encountered species on the potato crops. Around 58% of the sampled aphids were identified as *M. persicae*, followed by *A. gossypii* (14.79%), *A solani* (7.39%), *A. spiraecola* (5.55%), and *A. nerii* (4.07%).

The distribution of the aphid species on potato in the country is depicted in Fig. 1. Distribution of aphid species on potato was highly variable in various parts of the country. The major potato growing belt, *i.e.* Indo-Gangetic plains, witnessed highest aphid species diversity as compared to other parts of India. A total of thirteen out of fourteen species were recorded in this region. The most common among those were *M. persicae*, *A. gossypii*, *A. spiraecola*, *A. solani*, and *A. nerii*. *B. brassicae* was recorded on potato in Punjab, Uttar Pradesh, and West Bengal. Species like *R. rufiabdominale*, *A. fabae*, and *H. coriandri* were found in Jalandhar region of Punjab and in Gonda and in Modipuram regions of Uttar Pradesh, respectively, but in low numbers. Similarly, *L. erysimi*, *R. maidis*, and *A. craccivora* were explicitly observed in Gwalior region of Madhya Pradesh. *R. padi* was recorded from Hisar (Haryana). In western and north-eastern hills (Shimla, Shillong, and Kohima), the aphid species diversity observed on potato was low as compared to the Indo-Gangetic plains. In these regions, *M. persicae* and *A. gossypii* were the most common and dominant species. *A. solani*, *M. euphorbiae*, and *M. persicae* were recorded in Shimla and Solan districts of Himachal Pradesh. In north-western plains, plateau regions, and southern hills of India, the dominant aphid species was *M. persicae* followed by *A. gossypii*. The most dominant aphid vector, *M. persicae*, was distributed in all potato growing regions of India except Raipur. Molecular phylogenetic tree was constructed for the COI gene using the NJ method. The NJ tree showed that all sequences of 14 aphid species from potato unambiguously clustered into distinct clusters with already published sequences in GenBank (Fig. 3). The mean intra- and interspecific genetic distances of *A. spiraecola* were 1.4% (range, 0.0–6.60%) and 07.90% (range, 05.60–10.90%), respectively (Table 2). These values of intra- and interspecific genetic distances of *A. spiraecola* indicated slight overlap between them. For the rest of aphid species, genetic distance ranges indicated no overlap between intra- and inter-specific genetic distances among one another.

The collected aphid samples were tested for their viruliferous nature with respect to PVY^O and PLRV. PVY^O was detected in *M. persicae*, *A. gossypii*, *A. solani*, and *M. euphorbiae* among the 14 species tested, whereas PLRV was detected in *M. persicae* and *A. gossypii* only (Table 3). Averaged over all the locations, 39% and 21% of the collected individuals (n = 315) of *M. persicae* were found to carry PVY and PLRV, respectively. Similarly, 33% and 9% of the collected individuals (n = 80) of *A. gossypii* were found positive for PVY and PLRV, respectively. Among other species, 13% of the samples (n = 40) of *A. solani*, recorded from four locations, were viruliferous for PVY, while 25% of *M. euphorbiae* (n = 12), recorded only in Shimla hills, were found to carry PVY. Rest of the aphid species were found at lower densities on potato crops at various locations throughout the country; however, the viruses were not detected in them (Table 3; Fig. 1).

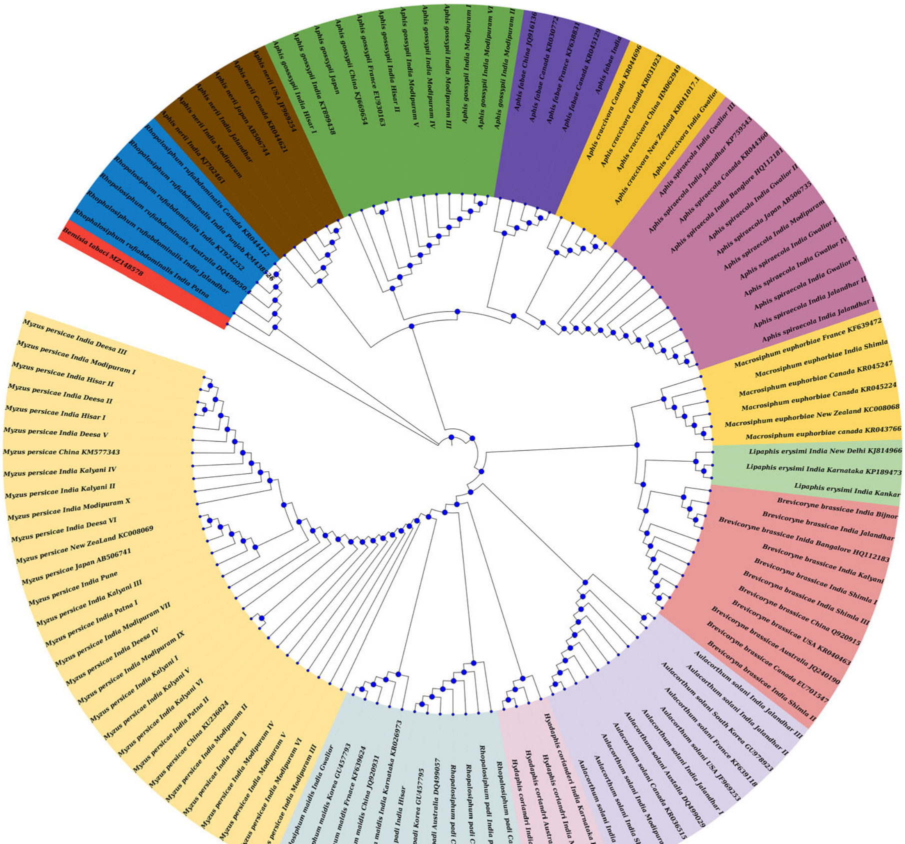

Fig. 3 Neighbor joining tree showing genetic relationships of aphid species and *Bemisia tabaci* as out-group collected from potato based on partial COI sequences. Different species coloured according to their taxa. Bootstrap values were obtained from a search with 1000 replicates.

Table 2 Intraspecific genetic distance (K2P) of potato aphids based on partial COI sequences that have two or more sequences of aphids with minimum, maximum, and average values

Species groups	No. of individuals	Intraspecific genetic distance (%)		
		Min.	Max.	Average
Aphis craccivora	5	0.00	2.60	0.10
Aphis fabae	5	0.00	0.00	0.00
Aphis gossypii	12	0.00	1.30	0.60
Aphis nerii	6	0.00	1.30	0.40
Aphis spiraecola	15	0.00	6.60	1.40
Aulacorthum solani	11	0.00	5.20	1.20
Brevicoryne brassicae	10	0.00	3.90	2.00
Lipaphis erysimi	4	0.00	0.60	0.40
Hyadaphis coriandri	3	0.00	1.30	0.60
Macrosiphum euphorbiae	6	0.00	0.00	0.00
Myzus persicae	30	0.00	2.60	0.70
Rhopalosiphum padi	7	0.00	0.60	0.40
Rhopalosiphum rufiabdominalis	6	0.00	0.60	0.40
Rhopalosiphum maidis	5	0.00	0.60	0.40

Discussion

Aphids were randomly sampled from potato crops across the major agro-ecological zones in India for characterization of species composition, geographical distribution, and viruliferousness with respect to PVY and PLRV. Morphological and molecular identification of the aphid samples confirmed the presence of fourteen species on potato in India. Among the 14 species recorded, *A. craccivora*, *A. gossypii*, *A. fabae*, *A. spiraecola*, *A. solani*, *M. euphorbiae*, *R. rufiabdominale*, and *M. persicae* are known as colonizing aphids of potato (Blackman and Eastop, 2000). The remaining species, which include *A. nerii*, *R. padi*, *R. maidis*, *H. coriandri*, *B. brassicae*, and *L. erysimi*, are considered non-colonizing on potato crops (Schroder and Krüger 2014; Mondal et al. 2016; Sukhoruchenko et al. 2019). Non-colonizing aphids do not primarily feed or breed on potato plants but feed on them transiently while searching for a more suitable host, while the colonizing species reproduce on potato plants and utilize them as food source for completion of life cycle (Davis and Radcliffe 2008). Due to non-persistent nature of PVY, the non-colonizing aphids are more important for its spread, while as for the transmission of persistent viruses like PLRV, colonizing aphids are more important (Ragsdale et al. 2001; Davis and Radcliffe 2008). Potato crops are generally visited by numerous non-colonizing aphids in large numbers and hence become the most important source for spread of PVY, in spite of their low virus transmission efficiency (Pelletier et al. 2012; Mondal et al. 2016).

More than 110 species of aphids are known to visit potato crops in the temperate areas of which 65 species have been demonstrated to transmit PVY and 12 species are known to transmit PLRV (Lacomme et al. 2017; Fox et al. 2017; Mondal et al. 2017). In contrast, fewer species of aphids were recorded from potato plants in the current

Table 3 Detection of PVY and PLRV in aphid samples collected from various locations using print capture PCR method

Serial No.	Species	Locations	No. of samples processed (*n*)	% tested positive for PVY	% tested positive for PLRV
1.	*Myzus persicae*	Himachal Pradesh	10	40.00	30.00
		Punjab	29	41.30	13.70
		Uttar Pradesh	35	40.00	14.20
		Madhya Pradesh	12	33.30	41.60
		Haryana	17	64.70	47.00
		Chhattisgarh	12	0.00	0.00
		Uttarakhand	14	28.57	28.57
		Gujarat	15	46.67	0.00
		Bihar	28	64.20	17.80
		West Bengal	17	29.40	35.20
		Maharashtra	23	60.86	30.40
		Odisha	12	41.60	0.00
		Karnataka	11	27.25	36.30
		Tamil Nadu	15	20.00	13.30
		Meghalaya	29	29.00	16.60
		Nagaland	12	66.67	41.60
		Sikkim	24	30.00	0.00
2.	*Aphis gossypii*	Punjab	23	30.43	8.70
		Uttar Pradesh	26	57.00	7.60
		Haryana	4*	25.00	0.00
		Chhattisgarh	4	0.00	0.00
		Bihar	8	25.00	12.50
		Karnataka	5*	20.00	0.00
		Meghalaya	4*	75.00	50.00
		Nagaland	6*	33.33	0.00
3.	*Aulacorthum solani*	Himachal Pradesh	20	25.00	0.00
		Punjab	10	20.00	0.00
		Uttar Pradesh	6	0.00	0.00
		Madhya Pradesh	4	0.00	0.00
4.	*Macrosiphum euphorbiae*	Himachal Pradesh	12	25.00	0.00
5.	*Aphis spiraecola*	Various	30	0.00	0.00
6.	*Rhopalosiphum rufiabdominale*	Punjab	5	0.00	0.00
7.	*Aphis nerii*	Various	22	0.00	0.00
8.	*Brevicoryne brassicae*	Various	13	0.00	0.00
9.	*Hyadaphis coriandri*	Uttar Pradesh	4	0.00	0.00
10.	*Aphis fabae*	Uttar Pradesh	7	0.00	0.00
11.	*Aphis craccivora*	Madhya Pradesh	2	0.00	0.00
12.	*Rhopalosiphum maidis*	Madhya Pradesh	3	0.00	0.00

Table 3 (continued)

Serial No.	Species	Locations	No. of samples processed (*n*)	% tested positive for PVY	% tested positive for PLRV
13.	*Lipaphis erysimi*	Madhya Pradesh	6	0.00	0.00
14.	*Rhopalosiphum padi*	Haryana	12	0.00	0.00

*The sampling size was very low (<6 samples). Detection of virus in one specimen counts for 20–25% of the virus contamination which needs thorough investigation by sampling large number of specimens in future

study as compared to other parts of the world. The primary reason for such a low diversity of aphids infesting potato crops in India could be that major acreage under potato cultivation in the country is located in the sub-tropical plains (Indo-Gangetic plains) where potato is cultivated during winter under short day conditions. Theoretically, the aphid activity would be lower during winters although the winters under subtropics could allow a considerable growth of aphid populations. Other than that, the cropping sequence and use of pesticides could affect the species composition and abundance of aphids in a particular location. Differential floristic composition in the

Table 4 Common potato viruses transmitted by various species of aphid

Serial No.	Aphid species	Potato viruses					Reference
		PVY	PLRV	PVA	PVM	PVS	
1	*Myzus persicae*	+	+	+	+	+	Tiwari et al. (2012)
2	*Aphis gossypii*	+	+				Kotzampigikis et al. (2008)
3	*Aulacorthum solani*	+	+				Verbeek et al. (2010); Kotzampigikis et al. (2008)
4	*Macrosiphum euphorbiae*	+	+*	+	+		Piron (1986); Radcliffe and Ragsdale (2002)
5	*Rhopalosiphum maidis*	+					Radcliffe and Ragsdale (2002)
6	*Aphis spiraecola*	+					Laird Jr and Dickson (1963)
7	*Rhopalosiphum rufiabdominale*	+					Chandla et al. (2004)
8	*Rhopalosiphum padi*	+					van Hoof (1977); Mello et al. (2011); Pelletier et al. (2012)
9	*Brevicoryne brassicae*	+					Boquel et al. (2011)
10	*Lipaphis erysimi*	+					Sigvald (1989)
11	*Aphis fabae*	+		+		+	Lin et al. (2009); Verbeek et al. (2010)
12	*Hyadaphis coriandri***						
13	*Aphis nerii***						
14	*Aphis craccivora*	+					Fereres et al. (1993)

*Poorly transmits PLRV; **status of viruliferousness is not yet known

sub-tropics would determine the species profile of aphids found there (Kilalo et al., 2013). Nonetheless, the species diversity is likely to be larger on potato, and more thorough studies would clarify the picture.

Historically, *M. persicae* was considered the only aphid of significance with respect to virus transmission and production of quality seed potatoes in India (Pushkarnath 1959, 1967). Over time, many more species were recorded from potato crops from different locations in India. For example, in addition to *M. persicae*, Pushkarnath (1959) reported the presence of *A. nastutii* in large numbers on potato. Bindra and Sekhon (1971) and Verma (1977) recorded seven and four species, respectively, on autumn crops in Punjab. Sekhon and Bindra (1979) reported four species from Kullu valley, whereas Kashyap and Verma (1982) reported two more species from Haryana. Misra and Agrawal (1987) summarized the information on biology and importance of twelve species of aphids from different parts of country. Lately, Kumara et al. (2017) reported five aphid species from potato crops in Karnataka. To the best of our knowledge, at least 17 species of aphids infesting potato crops in India are on record. Since most of the species recorded are established vectors of PVY or PLRV or both throughout the world, their role in virus spread under Indian growing conditions needs consideration.

Although most of the aphid species recorded in this study have a broad host range with worldwide distribution, only *M. persicae* and *A. gossypii* were recorded from most of the sampled sites. The remaining species were found to exhibit a narrow geographical distribution. This might be due to smaller sample size used and different cropping sequences and floristic composition for a particular location. One would expect a wider distribution of all the recorded aphid species, although with varying abundance of individual species with location.

In this study, the presence of PVY was confirmed in *M. persicae*, *A. gossypii*, *A. solani*, and *M. euphorbiae* whereas PLRV in *M. persicae* and *A. gossypii*, which could potentially transmit the respective viruses in potato crops. However, many of the species recorded in the current study are known as vectors of PVY and PLRV (Table 4). The transmission efficiencies of *A. solani*, *M. euphorbiae*, and *B. brassicae* have been determined under Indian conditions as 43.5%, 33.3%, 11.25%, 23.3%, and 21.8%, respectively, for PVY and PLRV (Sridhar et al. 2020; Sridhar et al. 2021a & 2021b). Although these aphids are not as efficient as *M. persicae*, they could definitely contribute to increased virus transmission under field conditions. Aphid vector pressure is directly proportional to virus incidence in potato wherein haulms are cut when vector pressure exceeds 20 aphids/compounding leaves as viral load exceeds tolerable limits (Pushkarnath 1967). Therefore, immigrating aphids can affect the health of seed potato crop. Non-colonizing aphids do not primarily feed or breed on potato plants but feed on them transiently while searching for a more suitable host, while the colonizing species reproduce on potato plants and utilize them as food source for completion of life cycle (Davis and Radcliffe 2008). Due to the non-persistent nature of PVY, the non-colonizing aphids are more important for its spread, while as for the transmission of persistent viruses like PLRV, colonizing aphids are more important (Ragsdale et al. 2001; Davis and Radcliffe 2008). High densities of adult populations of non-colonizing aphids can also contribute significantly in horizontal transmission of viruses by compensating for their weak transmission efficiency. Non-colonizing aphids have been shown to be major vectors of potyviruses in the past at Idaho. Hundreds of non-colonizing aphid species have been reported from

potato fields and tested for virus transmission ability. It has been assumed that, in the absence of potato-colonizing aphid species, PVY spread is caused by these non-colonizing aphid species (Boquel et al. 2012). Therefore, aphid vector dynamics and density play crucial role that determines the PVY and PLRV incidence in the field as well as overall health of seed and ware potato crop.

To conclude, the current vector-virus management programme for healthy seed potato production in India takes in to consideration the population build-up of *M. persicae* only. We found that there are many other species of aphids which could horizontally spread the dominant potato viruses, PVY and PLRV. Therefore, the relative importance of all the aphid species for virus transmission in seed potatoes needs to be worked out, and monitoring programmes focussing on all such species need to be started so that management decisions could be taken accordingly.

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Data Availability The data are available from the first author (JS) upon reasonable request.

Code Availability Not applicable

Author Contribution JS, SS, MN, and SKC conceived the problem. JS, AB, and VV planned the research. VV, AB, and MAS collected and curated the aphid samples. JS, VV, and NK did the molecular analysis. JS and MAS wrote the manuscript. All authors commented on the draft and approved it for publication.

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Consent for Publication All authors give consent for publication.

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