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Macrosiphum euphorbiae: A new aphid vector (Aphididae: Hemiptera) of PVY^{o} and PLRV on potato from north western hills of India

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Abstract
Vector borne viral diseases cause huge economic losses to many agricultural and horticultural crops across the world including India. More than forty aphid vectors are reported to infest potato globally including *Macrosiphum euphorbiae* (Thomas) of which *Myzus persicae* (Sulzer) has been referred as most efficient vector of viruses. Among viruses, *Potato virus Y*^{o} (PVY^{o}) and *Potato leafroll virus* (PLRV) are very significant in potato in India and are mainly transmitted by *M. persicae* and *Aphis gossypii* Glover. However, systematic investigation on occurrence of *M. euphorbiae* and its association in transmission of PVY^{o} and PLRV is yet to be determined in India. Hence, monitoring of *M. euphorbiae* in different potato growing regions of Shimla hills and its viruliferous nature pertinent to PVY^{o} and PLRV has been addressed here. The species identity was confirmed using mitochondrial COI gene markers and 99% similarity with reported sequences of database. Among the total samples of *M. euphorbiae*, 42, 40, 38, 30, 30 and 19% were found to be viruliferous to PVY^{o} and 32, 26, 20, 23, 15 and 14% for PLRV from Bemloe, Kanlog, Beakalty, Kufri, Gallu and Fagu respectively. In general, the incidence of PVY^{o} was more than PLRV in Shimla hills. The present study confirmed the occurrence of *M. euphorbiae* on potato and its viruliferous nature to PVY^{o} and PLRV in Shimla hills. Very interestingly, it was also found that *M. euphorbiae* could carry simultaneously multiple virus infection i.e. non–persistently PVY^{o} and Persistently PLRV.

Keywords: Potato aphid, *vector*, viruliferous, PVY^{o}, PLRV

Introduction
Potato aphid, *Macrosiphum euphorbiae* (Aphididae: Hemiptera) is an important polyphagous insect pest known to cause damage to many crops directly by sucking the plant sap and indirectly by transmitting infectious plant viruses which affect the quality and yield. *M. euphorbiae* has a broad host range including plants belong to Solanaceae and transmits number of plant viruses, and represents an aphid species of worldwide significance [1]. Its abundance and propensity to develop alatae makes this aphid species very important in viral epidemiology [2]. It act as vector of potato viruses and associated with decreased tuber yield.

In India, five important potato viruses namely PVY, PLRV, PVA, PVS, PVM are vectored by number of aphids [3] and at least eight viruses are transmitted by aphids in potato, *Solanum tuberosum* in world [5]. The vector borne viruses cause up to 20-50% yield loss in potato [5]. Potato virus Y^{o} (PVY^{o}) and *Potato leafroll virus* (PLRV) are the most important aphid transmitted viruses affecting potato crop. About 80 percent of problem of seed degeneration was associated with PVY and PLRV [6]. These two heterologous viruses are efficiently transmitted by colonizing vector, *Myzus persicae* (Sulzer) [1]. PVY is also less efficiently transmitted by several non-colonizing aphid vector, *Rhopalosiphum padi* (L.) [7, 8]. Zhu *et al*. in 2007 reported *M. euphorbiae* as good colonizer and potential vector of PVY [9]. Ragsdale *et al*. in 2001 observed that PVY was efficiently transmitted by *M. euphorbiae* ranging 4.0 and 29.0% [10]. Therefore, it is very important to monitor aphid species including *M. euphorbiae* associated with potato and their viruliferous nature pertinent to PVY^{o} and PLRV for the healthy seed potato production in hills. Based on the above said facts, the present study was conducted to monitor *M. euphorbiae* and to determine its viruliferous nature with respect to PVY^{o} and PLRV in Himachal Pradesh.

Materials and method

Monitoring and diagnostics of *M. euphorbiae*

The *M. euphorbiae* was monitored in potato fields of Kanlog, Bemloe, Fagu, Kufri, Gallu and Beakalty using standard procedures and a total of 268 samples were collected from these locations and preserved in 100% ethanol for morphological diagnostics and molecular characterization, and for determining viruliferous nature between April and August during 2014-2016. Representative potato leaf samples were also collected from the suspected plants based on visual symptoms. These aphid samples were identified on the basis of morphological description as described by Bishop *et al*. in 1982 and Joshi *et al.* in 2013 [11, 12]. Further aphid samples were sent to a nodal institute, National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka. In addition, aphid populations were identified using mitochondrial COI gene based marker LCO-1490; 5'-GGTCAACAAATCATAAAGATATTGG-3' and Antisense, HCO-2198; 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' [13, 14]. The total nucleic acid (DNA and RNA) was extracted by using Print capture method [15] and was quantified using Nano drop (Thermoscientific, Leon-Rot, Germany). A desired quantity (5 microliter) of nucleic acid containing DNA was used for PCR amplification using COI markers by following the standard protocols [15]. The amplified products were resolved on ethidium bromide and visualized in a gel documentation system. The PCR amplicons were purified and sequenced (Big dye® Applied Biosystem, UK) using standard protocols [16]. The sequences obtained were aligned, assembled and BLAST analyzed for identification of *M. euphorbiae*.

Determination of viruliferous nature of *M. euphorbiae*

The population of *M. euphorbiae* were tested for their viruliferous nature to PVY^{o} and PLRV. The nucleic acid (DNA and RNA) from individual aphid were used for viruliferous nature of *M. euphorbiae.* c-DNA synthesis and PCR was performed [16]. Similarly, the total RNA was extracted from individual leaf samples using the protocol of Sigma RNA extraction kit. c-DNA synthesis and PCR was performed according to Baswaraj *et al.* in 2014 [17]. The molecular markers used in this study for diagnostics are listed in table 1.

Table 1: The markers used for identifications of *M. euphorbiae* and for detection of PVY^{o} and PLRV in aphid as well as in potato plants

Primer Name	Polarity	Nucleotide Sequence 5'-3'	Amplicon size (bp)	GenBank Reference sequences (accession #)
LCO-1490	Sense	5'-GGTCAACAAATCATAAAGATATTGG-3'	658	Rebijith *et al.* (2013)
HCO-2198	Antisense	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'		
PVY-FCP	Sense	5'-ACGTGGTATGAGGCAGTGCGGA-3'	380	Venkateswarlu *et al*. (2016)
PVY-FCP	Antisense	5'-ATGTGCGCTTCCCTAGCCCTCA-3'		
PLRV-FCP	Sense	5'-CTAACAGAGTTCAGCCAGTGGTTA-3'	492	Bawaraj *et al*. (2014) Venkateswarlu *et al*. (2016)
PLRV-RCP	Antisense	5'-CGGTATCTGAAGATTTTCCATTTC-3'		

Results

Monitoring and diagnostics of *M. euphorbiae*

A total of 268 aphids were collected from six potato growing areas (112 from Bemloe, 30 from Kanlog, 42 from Fagu, 33 from Kufri, 30 from Gallu and 21 from Beakalty). The field collected population of *M. euphorbiae* were identified by morphological and molecular markers. The major key diagnostic characters of *M. euphorbiae* are the body is pear-shaped reaching about four millimetres long. The antennal parameters like dark at the joints between the segments and are longer than the body; legs longer than in other aphids, pale green but darker at the apices; the siphunculi are pale coloured, cylindrical with dark tips and operculi, and are about one third the length of the body. The tail is sword-shaped and bears 6 to 12 hairs and is much shorter than the siphunculi (Fig. 1). Morphological characters was further authenticated by using the molecular diagnostics (Fig. 2). The sequences generated in this study (GenBank accession no. KY613938) displayed 98-99% identity with the reference sequences of NCBI.

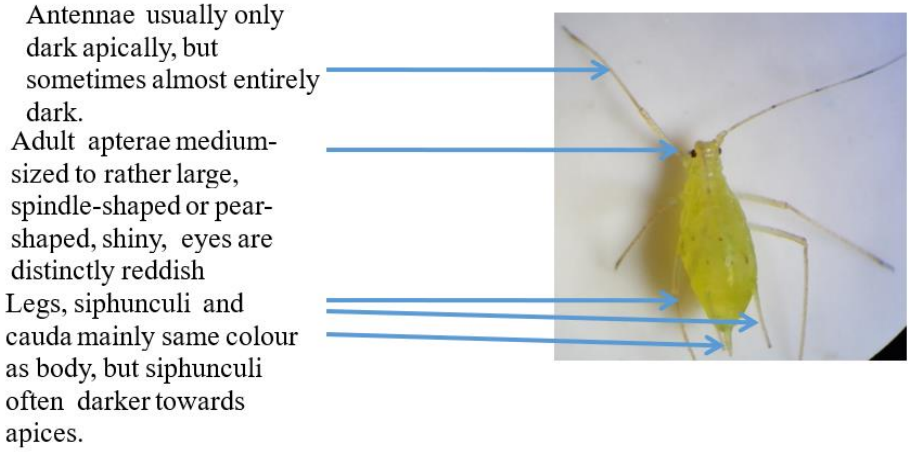

Fig 1: Description of morphological key characters of *M. euphorbiae*

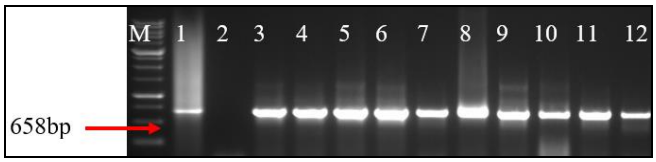

Fig 2: PCR amplification of mt COI gene of *M. euphorbiae*. Marker 1kb, Lane 1- +ve control, Lane 2- negative control, Lane 3-7- Shimla samples, Lane 8-12-Kufri samples.

Determination of viruliferous nature of *M. euphorbiae*.

A total of 268 samples were collected from six potato growing areas i.e. 112 from Bemloe, 30 from Kanlog, 42 from Fagu, 33 from Kufri, 30 from Gallu and 21 from Beakalty were processed to determine viruliferous nature with respect to PVY^{o} and PLRV. The results revealed that *M. euphorbiae* were found to be positive for multivirus infection of PVY^{o} and PLRV. A maximum of 42% PVY^{o} infection was reported in *M. euphorbiae* population from Bemloe followed by 30% and 19% in Kufri and Fagu, respectively (Table 2 and Fig 3) and maximum of 32% PLRV infection from Shimla followed by 15 and 14% from Kufri and Fagu, respectively (3a). Similar results were obtained in case of potato plants samples. A total of 130 leaf samples were processed for the detection of PVY^{o} and PLRV. The results revealed that 40 samples were found to be positive for infection of viruses PVY^{o} and 31 for PLRV (Table 2). A maximum of 40% PVY infection was reported on from Kufri followed by 36.66% and 30% in Shimla and Fagu, respectively (Table 2 and Fig 4). PLRV virus was also detected with maximum of 30% from Kufri, 23.33% from Shimla and minimum of 10% from Fagu (Fig.5). The transmission studies under controlled conditions at CPRI, Shimla were also confirmed *M. euphorbiae* as a good vector of PVY^{o} and PLRV (unpublished data).

Fig 3: Detection of PVY^{o} in *M. euphorbiae.* Marker 100 bp, Lane 1- +ve control (380 bp), Lane 2-7- Shimla samples of *M. euphorbiae* 8- Healthy control.

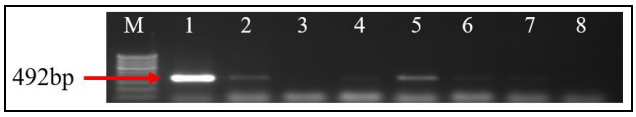

Fig 3a: Detection of PLRV in *M. euphorbiae*. Marker 100 bp, Lane 1- +ve control, Lane 2- negative control, Lane 3-8- *M. euphorbiae* samples of Shimla.

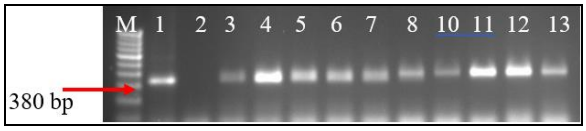

Fig 4: Detection of PVY^{o}in field samples of potato. Marker 100 bp, Lane 1- +ve control, Lane 2- negative control, Lane 3-7- potatosamples of Shimla, Lane 8-12- potato samples of Kufri.

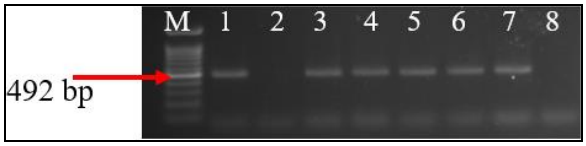

Fig 5: Detection of PLRV in field samples of potato. Marker 100 bp, Lane 1- +ve control, Lane 2- negative control, Lane 3-8- potato samples of Shimla.

Table 2: Determination of virulifeorus nature of *M. euphorbiae* with respect to PVY^{o} and PLRV

Location	Samples (Nos)	Viruliferous populations (Nos and %)	
		PVY	PLRV
Kufri	33	10 (30)	5 (15)
Fagu	42	8 (19)	6 (14)
Bemloe	112	48 (42)	36 (32)
Kanlog	30	12(40)	8 (26)
Gallu	30	9(30)	6(20)
Beakalty	21	8(38)	5(23)

Discussion

The identification of virus vector, *M. euphorbiae* using morphological and molecular diagnostics, and its association in transmission of PVY^{o} and PLRV was addressed in this study. Aphid vectors play a crucial role in transmitting dreadful plant viruses that cause huge economic losses to crops. About 190 aphid species are known to transmit plant viruses, with many species capable of transmitting more than one virus species [18, 19] including potato viruses. In India, a total 13 aphid species were recorded on potato including *M. euphorbiae* and their relative importance as vectors of PVY^{o} and PLRV have not been worked out [20]. In this study, the populations of *M. euphorbiae* were monitored in Shimla hills and species identity was confirmed using morphological and molecular diagnostic techniques. The abundance and distribution of *M. euphorbiae* was low in Shimla hills. Based on the intensive surveys on aphid monitoring on the potato in the country, very interestingly *M. euphorbiae* was recorded only in Himachal Pradesh (unpublished data). The population reached its peak on potato during first and second week of August. Based on the mitochondrial COI gene sequences, *M. euphorbiae* populations of India showed 99-100% similarity with populations of USA, Canada, New Zealand, Australia.

With regard to transmission, our results showed that *M. euphorbiae* was highly viruliferous to PVY^{o} and PLRV under field conditions. One third of random field populations of *M. euphorbiae* were carrying the inoculum of PVY indicating that it is a viruliferous vector. It has been reported in previous studies that *M. persicae* is the most efficient aphid vector of PVY in the world [6, 21]. This study clearly shows vector profile is getting changed over the years in north western hills. Besides this, laboratory studies also proved and confirmed that *M. euphorbiae* could transmit PVY^{o}. It was found that a maximum of 42% of *M. euphorbiae* could carry inculum of PVY and minimum of 19% at Shimla hills. Previously, Zhu *et al.* in 2007 also reported *M. euphorbiae* as a good colonizer and potential vector of PVY^{o} [9]. It is a non-persistent, stylet-borne virus and a vector can acquire and transmit the virus in few minutes. Our results are supported by Ragsdale *et al*. in 2001 [10], transmission efficiency of PVY by *M. euphorbiae* between 4.0 and 29.0%. However, PVY^{o} is transmitted in a non-persistent manner by aphid vectors that differ in their transmission efficiency [22]. In our study, the plants samples collected from potato fields of Shimla, Fagu and Kufri were also found positive for PVY^{o} infection and caused maximum infection of PVY^{o} on potato plant samples from Kufri followed by 36.66% and 30% in Shimla and Fagu, respectively. It was in accordance with the results recorded on potato yield losses of up to 80% in United States [23].

Results also revealed that *M. Euphorbiae* not only transmit PVY^{o} but also transmit PLRV. PLRV is persistent and circulative in *M. euphorbiae* and recorded 32% of PLRV

infection from Shimla. In the earlier reports, PLRV is transmitted almost exclusively by *M. Persicae* and *M. euphorbiae* [1]. Khaled *et al*. in 2018 reported a highest transmission potential of 80% for *M. euphorbiae* which clearly indicate that it an efficient vector of PLRV [24]. They also reported for the first time, its transmission potential was at par with *M. persiace* (90%). However, it is in contrast with the findings of Robert (1971), who found a low efficiency of this species compared to *M. persicae* [25]. In addition, it was also reported that *M. euphorbiae* could successfully transmit PVA and PVM globally [1]. The present study reports that *M. euphorbiae* can transmit and act as vector of PVY^{o} and PLRV in Shimla hills.

Conclusion

The present study reports the occurrence and colonization of *M. euphorbiae* on potato in Shimla hills of Himachal Pradesh. This study also confirms that *M. euphorbiae* is vector of PVY^{o} and PLRV in Shimla hills. It is suggested that populations of *M. euphorbiae* need to be considered for calculating vector thresholds for producing healthy seed potatoes.

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