

MAPPING OF APHID SPECIES ASSOCIATED WITH POTATO IN INDIA USING MORPHOLOGICAL AND MOLECULAR TAXONOMIC APPROACHES

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ABSTRACT: Field surveys were carried out from 2011 to 2015 in different potato growing areas of India for collecting aphid species infesting potato and the aphid species were identified using molecular and morphological approaches. Thirteen species of aphids were identified from different regions of the country. *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover were the most common species and high population of these aphids was recorded on potato during first week of February in different parts of Uttar Pradesh. *Aulacorthum solani* (Kaltenbach) and *Brachycaudus helichrysi* (Kaltenbach) are being reported for the first time from India from Faizabad region of Uttar Pradesh. The black bean aphid, *Aphis fabae* Scopoli was identified from Manikpur and Tanda while as green citrus aphid, *Aphis spiraecola* Patch, oleander aphid, *Aphis nerii* Rothschild and coriander aphid, *Hydaphis coriandri* (Das) apart from *Myzus persicae* and *Aphis gossypii* were identified from Meerut during October to December. Rice root aphid, *Rhopalosiphum rufiabdominalis* (Sasaki), *Myzus persicae*, *Aphis gossypii* and *Aphis spiraecola* were identified from Jalandhar (Punjab). These findings need to be taken into consideration in the pest management for healthy seed potato production.

KEYWORDS: Aphids, potato, morphological taxonomy, cytochrome oxidase-1, potato virus

INTRODUCTION

There are about 4700 species of aphididae in the world. Of these, 4500 species of aphids have been reported from crop plants, of which 100 species have successfully exploited the crop environment to the extent that they are of significant economic importance (Joshi *et al.*, 2013). Aphids are economically important polyphagous pests known to cause damage to many crops directly by sucking the plant sap, affecting yields and indirectly by transmitting the infectious plant viruses thus affecting both quality and yield. An extensive field survey was carried out for identification of aphid free period in India during 1969 (Chandla *et al.*, 2004). With the increasing temperature due to global climate change, change in cropping sequence, introduction of potato in new areas, diversification of potato

cultivars and use of high dose of chemical fertilizers, number and incidence of aphids is increased many folds on potato. The problem is further aggravated due to the development of resistance against conventional pesticides being used by the farmers (Bhatnagar *et al.*, 2012).

Therefore, the seriousness of aphids demands the quick identification of new aphid species infesting potato and their viruliferous nature for their timely management.

MATERIALS AND METHODS

Extensive field surveys were carried out in the potato growing states of India including Uttar Pradesh (Meerut, Muzaffarnagar, Kanpur, Sultanpur, Faizabad, Hapur, Bulandshahr, Aligarh, Hathras, Agra, Etawah, Firozabad, Mainpuri, Kannauj, Farrukhabad, Fatehpur,

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Lucknow, Raibareilly, Bijnor, Bagpat), Chhattisgarh (Raipur, Damtari, Kankar and Jagdalpur), Madhya Pradesh (Gwalior and Indore), West Bengal (Kalyani and Bardwan), Himachal Pradesh (Kufri, Solan and Shimla), Punjab (Jalandhar), Bihar (Patana), Maharashtra (Pune), Haryana (Hisar), Gujarat (Deesa), Meghalaya (Shillong, Makriai hills and Nangkynrih), Arunachal Pradesh, Nagaland and Sikkim for collection of aphid samples associated with potato from 2011 to 2015. Beside this, yellow water traps were also installed at four locations for collection of aphid species in the vicinity of (Modipuram) Meerut which is major potato belt. Aphid samples were collected in small plastic vials and preserved in 70% alcohol for correct identification at species level. Morphological identification of species was carried out at NBAIR, Bangalore and Division of Entomology, IARI, New Delhi. The aphids were also identified using species specific primers and universal primers (658bp) specific to cytochrome oxidase 1 gene for molecular identification. All the steps required for total genomic DNA extraction, PCR amplification employing mitochondrial COI universal primers, gel elution, cycle sequencing, purification and sequencing were carried out as per the procedure suggested by (Sridhar *et al.* 2016).

RESULTS AND DISCUSSION

Thirteen species of aphids were identified from different regions of the country on potato crop. The peach-potato aphid, *Myzus persicae* (Sulzer) and the cotton aphid, *Aphis gossypii* Glover were the most common species and high population (31-255/100 compound leaves) of these aphids was recorded on potato during first week of February in different parts of Uttar Pradesh. *Aulacorthum solani* (Kaltenbach) and *Brachycaudus helichrysi* (Kaltenbach) were reported for the first time on potato from India from Faizabad region of Uttar Pradesh. The complete list of the identified aphid species

along with their distribution is given in **Table 1** and **Figures 1 to 6**.

Myzus persicae from Gonda, *Aphis gossypii* from Gonda, Mashoda and Faizabad region of Uttar Pradesh and black bean aphid, *Aphis fabae* Scopoli was identified from Manikpur and Tanda of UP. The aphid samples collected from Meerut during October to December were identified as green citrus aphid, *Aphis spiraeicola* Patch, oleander aphid, *Aphis nerii* Rothschild and coriander aphid, *Hydaphis coriandri* (Das) apart from *Myzus persicae* and *Aphis gossypii*. Rice root aphid, *Rhopalosiphum rufiabdominalis* (Sasaki), *Myzus persicae*, *Aphis gossypii* and *Aphis spiraeicola* were identified from Jalandhar (Punjab). Aphids from Meghalaya (Shillong, Makriai hills and Nangkynrih), Arunachal Pradesh, Nagaland, Sikkim and HP (Kufri and Solan) were identified as *Myzus persicae* and *Aphis gossypii*. The diagnostic morphological characters of the aphid species are described as follows.

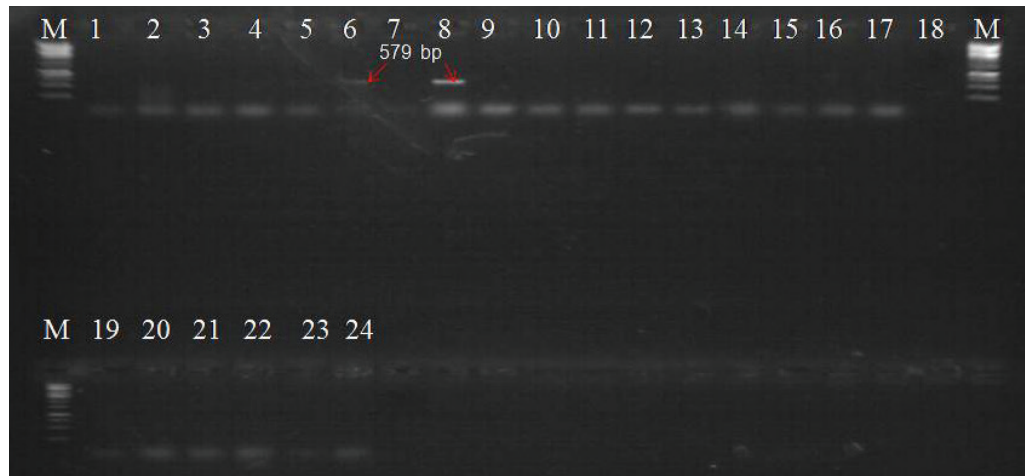
1. *Myzus persicae* (Sulzer)

Adult apterae small to medium-sized, whitish green, pale-yellow-green, grey-green, mid-green, pink, red or almost black, uniformly coloured, not shiny, Alatae have a black central dorsal patch on the abdomen, Apterae and alatae 1.2-2.1 mm. Head dorsally spinulose or warty either entirely or leaving a broad central portion free, ventrally also spinulose, with hardly or weakly developed median prominence but moderately to well-developed lateral frontal tubercles with the inner margin converging to diverging, Antennae 0.62 times as long as body, Processus terminalis three times or more than three times as long as the base of the last antennal segment, Siphunculi 0.27 times as long as body, Siphunculi almost twice the length of cauda, ultimate rostral segment as long as ht^2 as described by (Blackman and Eastop, 1984, Joshi *et al.*, 2013).

Table 1. Distribution of aphid species associated with potato in India

S. No.	Aphid Species		Host Plants (cultivated & wild)	Distribution in India	Virus Association
	Common Name	Scientific Name			
1.	Green peach aphid	<i>Myzus persicae</i> (Sulzer)	400*	Uttar Pradesh (Modipuram)	Potato Leaf Roll and Pot y virus
2.	Cotton aphid	<i>Aphis gossypii</i> Glover	100*	Uttar Pradesh (Modipuram)	PVY
3.	Bean aphid	<i>Aphis fabae</i> Scopoli	200*	Uttar Pradesh (Faizabad)	PVY
4.	Root aphid	<i>Rhopalosiphum rufiabdominalis</i> (Sasaki)	100*	North-east, Bihar	PVY
5.	Tuber aphid	<i>Rhopalosiphum latysiphon</i> (Davidson)	125*	North-east, Bihar	PVY
6.	Green citrus aphid	<i>Aphis spiraecola</i> Patch	100	Uttar Pradesh (Modipuram)	Not Known
7.	Oleander aphid	<i>Aphis nerii</i> Boyer de	100	Uttar Pradesh (Modipuram)	Not Known
8.	Coriander aphid	<i>Hydaphis coriandri</i> (Das)	75	Uttar Pradesh (Modipuram)	Not Known
9.	Foxglove aphid	<i>Aulacorthum solani</i> (Katterbach)	124*	Himachal Pradesh (Kufri, Solan) Uttar Pradesh (Pithola), Bihar (Patana), Gujrat (Deeca)	PVY
10.	Leaf curling plum aphid	<i>Brachycaudus helichrysi</i> (Kaltenbach)	128*	West Bengal (Nadia, Kalyani), Bihar (Dholi)	Not Known
11.	Potato aphid	<i>Macrosiphum euphorbiae</i> Thomas	69*	Himachal Pradesh (Shimla)	Potato Leaf roll and Leaf Speckling Virus
12.	Cabbage aphid	<i>Brevicoryne brassicae</i> (Linnaeus)	102*	West Bengal, Uttar Pradesh (Modipuram), Punjab	PVY
13.	Mustard aphid	<i>Lipaphis erysimi</i> (Kaltenbach)	156	Chhattishgarh (Kankar)	PVY

*Potato

**Fig 1. Print capture method of aphid identification**

Myzus Persicae : M-1 kb ladder, Lane1-water control, 2, 3-Patna, 4-Pasighat, 5-Jalandhar, 6-Gwalior, 7-Pune, 8-Uttarakhand, 9-MP, 10-Kanpur, 11-Bihar and 12-Haryana

Aphis gossypii : M-1 kb ladder, Lane13-water control, 14, 15-Patna, 16-Pasighat, 17-Jalandhar, 18-Gwalior, 19-Pune, 20-Uttarakhand, 21-MP, 22-Kanpur, 23-Bihar and 24-Haryana

2. *Aphis gossypii* (Glover)

Apterae are very variable in colour, Large specimens are dark green, almost black, but the adults produced in crowded colonies at high temperature may be less than 1 mm long

and very pale yellow to almost white, Most commonly the nymphs are light green mottled with darker green, with dark siphunculi and a pale or dusky pale cauda. Cauda processes terminals less than twice the length of base

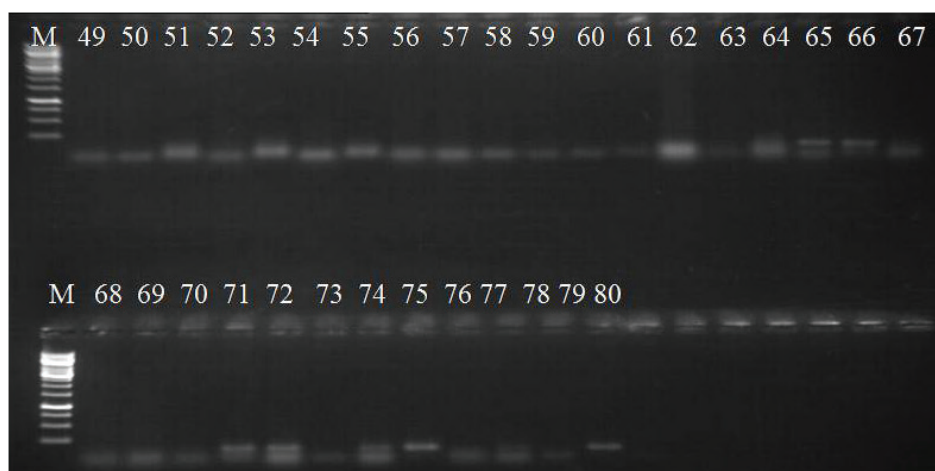


Fig 2. *Aphis fabae* and *Macrosiphum euphorbiae* primers amplified at different temp.

Distribution of Aphids- *A fabae* 1F1R, M-1 kb ladder, 41-water control, 42-Pithala, 43-Mashoda, 44-Faizabad, 45-Bawan, 46-Gonda, 47-Mallikpur, 48-Tendna, 49-Kumarganj 50-Dabhasomar & 51-Chandapur, other locations 52-Patna, 53-Pasighat, 54-Jalandhar, 55-Gwalior, 56-Pune, 57-Uttarakhand, 58-MP, 59-Kanpur and 60-Bihar.

Distribution of Aphids- *Macrosiphum euphorbiae* 1F1R, M-1 kb ladder, Lane 61-water control, 62-Pithala, 63-Mashoda, 64-Faizabad, 65-Bawan, 66-Gonda, 67-Mallikpur, 68-Tendna, 69-Kumarganj, 70-Dabhasomar & 71-Chandapur, other locations 72-Patna, 73-Pasighat, 74-Jalandhar, 75-Gwalior, 76-Pune, 77-Uttarakhand, 78-MP, 79-Kanpur and 80-Bihar.

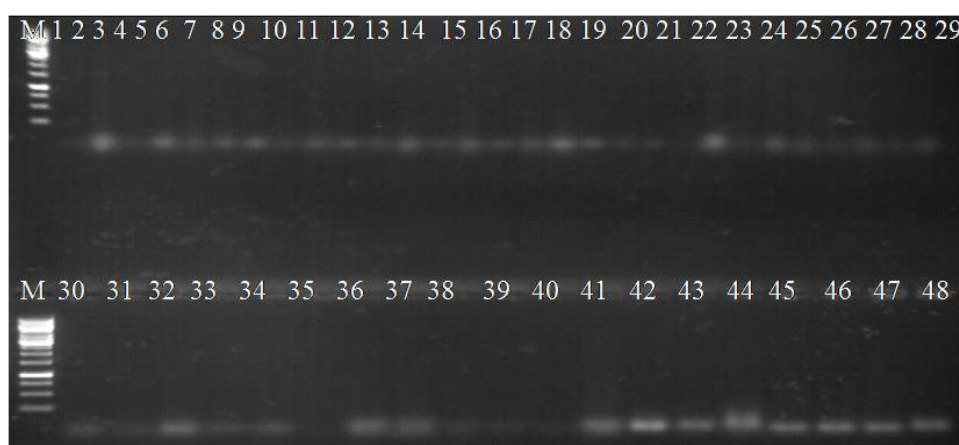


Fig. 3. *Aphids-Myzus persicae* and *Aphis gossypii*

Distribution of Aphids- *Myzus persicae* 1F4R, M-1 kb ladder, Lane1-water control, 2-Pithala, 3-Mashoda, 4-Faizabad 5-Bawan, 6-Gonda, 7-Mallikpur, 8-Tendna, 9-Kumarganj, 10-Dabhasomar & 11-Chandapur, other locations 12-Patna, 13-Pasighat, 14-Jalandhar, 15-Gwalior, 16-Pune, 17-Uttarakhand, 18-MP, 19-Kanpur and 20-Bihar.

Distribution of Aphids- *Aphis gossypii* 3F3R, M-1 kb ladder, Lane21-water control, 22-Pithala, 23-Mashoda, 24-Faizabad, 25-Bawan, 26-Gonda, 27-Mallikpur, 28-Tendna, 29-Kumarganj, 30-Dabhasomar & 31-Chandapur, other locations 32-Patna, 33-Pasighat, 34-Jalandhar, 35-Gwalior, 36-Pune, 37-Uttarakhand, 38-MP, 39-Kanpur and 40-Bihar.

of last antennal segment and always shorter than antennal segment III, Cauda with 4-7 hairs, often dusky, but distinctly paler than siphunculi (Blackman and Eastop, 1984, Chandla *et al.*, 2004, Joshi *et al.*, 2013).

3. *Aphis fabae* (Scopoli)

Young colonies consist of matt black aphids on young shoots, older colonies spread over most of aerial parts of plant with many

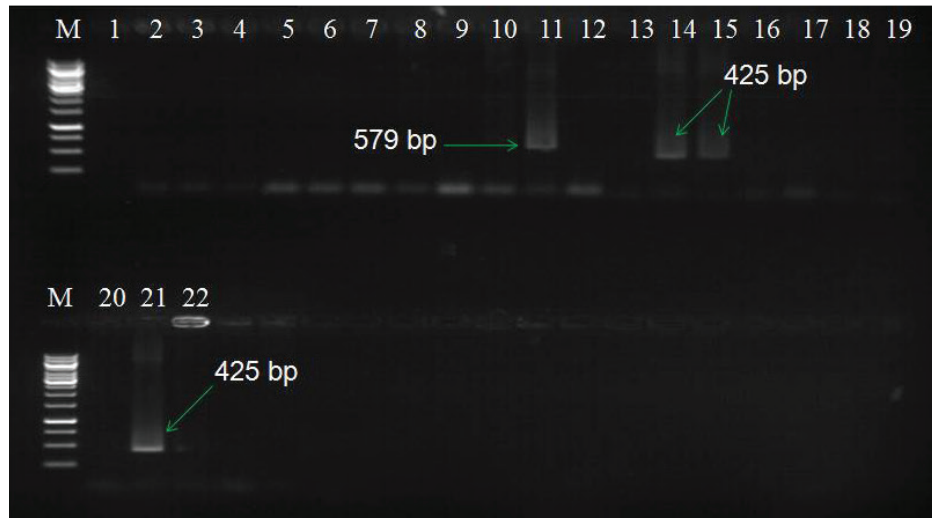


Fig. 4. Aphid species identification- *Myzus persicae* and *Aphis gossypii*

Distribution of Aphids- *Myzus persicae* 1F4R, M-1 kb ladder, Lane1-water control, 2-Positive control (*Myzus persicae*) 3-Pithala, 4-Gonda, 5-Faizabad, 6-Mashoda, 7-Bawan, 8-Faizabad, 9-Tendna, 10-Kumarganj, 11-Dabhasomar & 12-Chandapur, *Aphis gossypii* 3 F3R 13-Pithala, 14-Gonda, 15-Faizabad, 16-Mashoda, 17 Bawan, 18-Faizabad, 19-Tendna, 20-Kumarganj 21-Dabhasomar & 22-Chandapur.

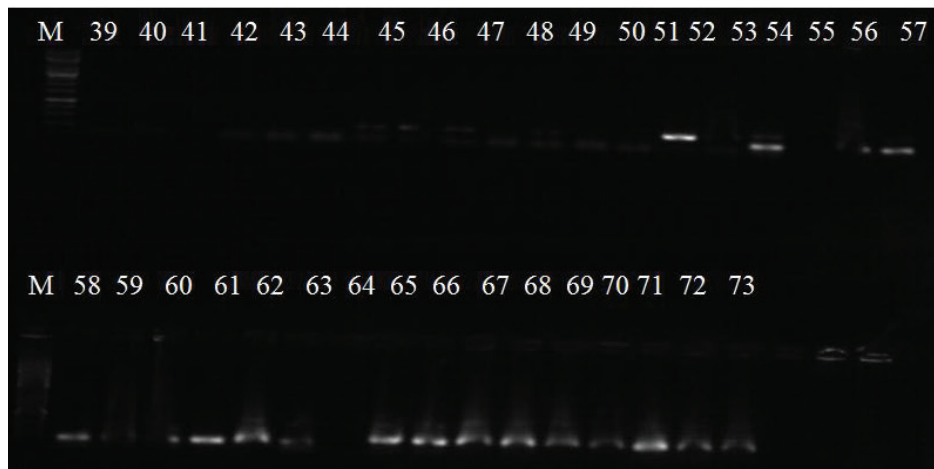


Fig. 5. Identification of Aphid species: *Lipaphis erysimi* and *Brevicoryne brassicae*

Distribution of Aphids- *Lipaphis erysimi* 1F1R, M-1 kb ladder, Lane 43-water control, 44-Pithala, 45-Gonda, 46-Faizabad, 47-Mashoda, 48-Bawan, 49-Malikipur, 50-Tendna, 51-Kumarganj, 52-Dabhasomar & 53-Chandapur *Lipaphis erysimi* 2 F3R 54-Pithala, 55-Gonda, 56-Faizabad, 57-Mashoda, 58-Bawan, 59-Faizabad, 60-Tendna, 61-Kumarganj, 62-Dabhasomar & 63-Chandapur. *Brevicoryne brassicae* 1F1R 64-Pithala, 65-Gonda, 66-Faizabad, 67-Mashoda, 68-Bawan, 69-Malikipur, 70-Tendna, 71-Kumarganj, 72-Dabhasomar & 73-Chandapur.

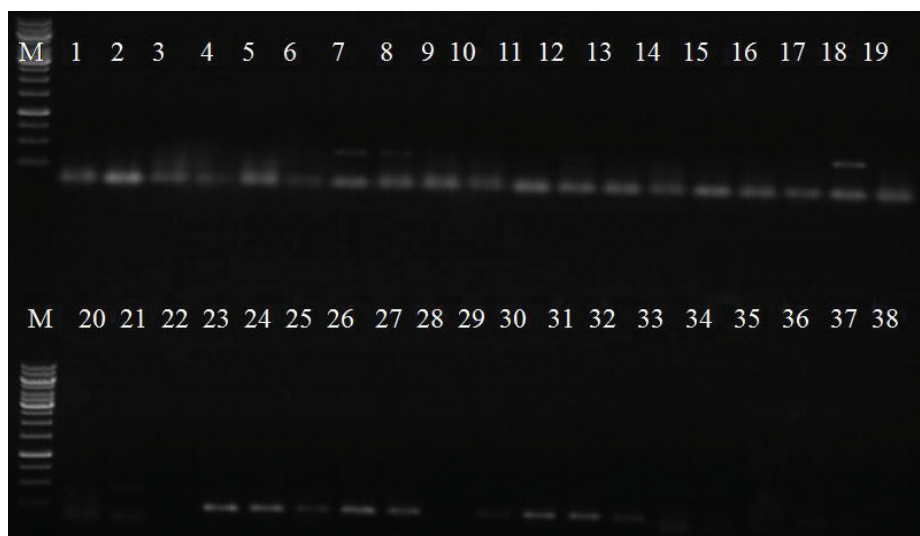


Fig. 6. Identification of Aphid species: *Aphis fabae* and *Macrosiphum euphorbiae*

Distribution of Aphids- *Aphis fabae* 1F1R, M-1 kb ladder, Lane1-water control, 2-Pithala, 3-Gonda, 4-Faizabad, 5-Mashoda, 6-Bawan, 7-Malikpur, 8-Tendna, 9-Kumarganj, 10-Dabhasomar & 11-Chandapur, *Aphis fabae* 3 F3R 12-Pithala, 13-Gonda, 14-Faizabad, 15-Mashoda, 16-Bawan, 17-Malikpur, 18-Tendna, 19-Kumarganj, 20-Dabhasomar & 21-Chandapur. *Macrosiphum euphorbiae* 1F1R Lane22-water control, 23-Pithala, 24-Gonda, 25-Faizabad, 26-Mashoda, 27-Bawan, 28-Faizabad, 29-Tendna, 30-Kumarganj, 31-Dabhasomar and 32-Chandapur. *Macrosiphum euphorbiae* 2F2R 33-Pithala, 34-Gonda, 35-Faizabad, 36-Mashoda, 37-Bawan, 38-Faizabad, 39-Tendna, 40-Kumarganj, 41-Dabhasomar & 42-Chandapur.

individuals developing white wax marking, regularly ant-attended, Apteræ 1.5-3.1 mm, alatae 1.3-2.6 mm. Cauda dark, Abdominal dorsum with small dark sclerites at least laterally on segments anterior to siphunculi, Transverse dark bands on tergite 7 and 8, Cauda without clear constriction, thumb shaped with 10-13 hairs (Blackman and Eastop, 1984, Verma and Chandla, 1999, Joshi *et al.*, 2013).

4. *Rhopalosiphum rufiabdominalis* (Sasaki)

Apteræ dark-green or olive with usually a reddish area is at the posterior end of abdomen between and around siphunculi. The abdomen of the alate is similarly colored. The aphid is less hairy as compared to *R. padi*. Abdominal segment VIII with 4-8 dorsal hairs, Antennae of apteræ normally 5- rarely six- segmented, Antennal hairs long and fine, much longer than maximum diameters of segments, Hairs on the body and legs long and fine, similar to antennal hairs (Blackman

and Eastop, 1984, Verma and Chandla, 1999, Joshi *et al.*, 2013).

5. *Rhopalosiphoninus latysiphon* (Davidson)

Siphunculi bagpipe-like with a few transversely cells just before the well developed flange, large antennal tubercles with parallel inner sides, processes terminalis, usually shorter than antennal segment III, dorsum of abdomen in apteræ pale, either smooth or wrinkled or with hexagonal spinular reticulation, in alatae with spinopleural patches sometimes fused together and with marginal patches in all segments upto segment VII, in alatae antennal segment III with 20-25 secondary rhinaria whitish, yellowish to black, body broadly oval on sprouts (Blackman and Eastop, 1984, Verma and Chandla, 1999, Joshi *et al.*, 2013).

6. *Aphis spiraeicola* (Patch)

Small aphid, often curling and distorting leaves near stem apices of host plants, or

on flower heads, and usually ant-attended, Body bright greenish yellow or yellowish green to apple green, head brown, Legs and antennae mainly pale but siphunculi and cauda dark brown to black, Alatae have head and thorax dark brown, abdomen yellowish green with a dusky lateral patch on each segment. Antenna almost half the length of the body, Siphunculi and cauda dark, Cauda usually with a constriction, with 8-12 hairs, Abdominal tergum smooth, without any patch (Blackman and Eastop, 2006, Verma and Chandla, 1999, Joshi *et al.*, 2013).

7. *Aphis nerii* (Boyer de)

It is thought that the oleander aphid is an obligate parthenogenetic species; thus the adult aphids are all female and males do not occur in the wild. Adult females may be winged or wingless. The winged adult females (alata) are yellow and black with dark wing veins while the wingless forms (apterae) are yellow with black cornicles, antennae, legs, and cauda (tip of abdomen). Nymphs are similar to apterae in appearance except that they are smaller. Size ranges from 1.5 to 2.6 mm in length (Blackman and Eastop, 2006, Verma and Chandla, 1999, Joshi *et al.*, 2013).

8. *Hydaphis coriandri* (Das)

Coriander aphids are yellow-green in color, dusted with greyish wax. They have short, dusky, slightly swollen, siphunculi (or cornicles) that are about twice as long as wide. They form dense and often damaging colonies on leaves, heads, and stems of their host plants. Several other species colonize the common hosts of the coriander aphid. These include various *Aphis* spp., like the green peach aphid (*Myzus persicae* (Sulzer)), and the rice root aphid (*Rhopalosiphum rufiabdominalis* (Sasaki)). Both green peach aphids and the *Aphis* spp. have siphunculi that is much longer than twice their diameter

(Blackman and Eastop, 2006, Kumar, 1994, Joshi *et al.*, 2013).

9. *Aulacorthum solani* (Kaltenbach)

Apterous adult females vary in color from pale green to yellow (there may be 2 distinct color morphs) with a body length of 1.8-3.0 mm, making them larger than *M. persicae*. They have 6-segmented antennae, with dark apices. The antennal tubercles are well developed and their inner faces are parallel (vs. *M. persicae*, which has convergent inner faces). The siphunculi are pale with dark tips, gradually tapering but with a distinct large apical flange and 2 rows of reticulations. Typically, dark green blotches can be seen near the base of the siphunculi. The cauda is pale and elongate. Alate females are yellow-green with a brown head, and a dark thorax and abdomen with pale to dark transverse bands. The body shape is similar to wingless females, but is 2.0-3.0 mm long (Miller and Stoetzel 1997). In practice, the foxglove aphid is easily distinguished from other aphid species due its i) larger body size than green peach or melon aphid, ii) dark joints of the antennae and legs, iii) parallel antennal tubercles, iv) the usual presence of darker green patches at the base of the siphunculi (Jandricic *et al.*, 2010), and v) overall “shiny” appearance (vs. the more matte appearance of *M. persicae*) (Murphy and Shipp, 2006). Behaviorally, *A. solani* is also known to engage in defensive dropping behavior (Jandricic *et al.*, 2010 Joshi *et al.*, 2013), which also distinguishes it from other floriculture pest aphids.

10. *Brachycaudus helichrysi* (Kaltenbach)

The adult aptera on the primary host is variable in colour ranging from yellow to green to brown, often shiny with slight wax dusting. On the secondary hosts they can be yellow, green or almost white to pinkish. The antennae are shorter than the body with

dusky tips. The dorsum of the abdomen is without a black shield. The siphunculi are pale, tapered and short-0.8-2.0 times the length of the cauda. The cauda is pale, short and blunt. The body length of apterae is 0.9- 2.0 mm.

11. *Macrosiphum euphorbiae* (Thomas)

Adult apterae medium-sized to rather large, spindle-shaped or pear-shaped, shiny, eyes are distinctly reddish, Legs, siphunculi and cauda mainly same colour as body, but siphunculi often darker towards apices, Antennae usually only dark apically, but sometimes almost entirely dark, Immature rather long-bodied, paler than adults with a dark spinal stripe and a light dusting of whitish-grey wax, Alatae have pale greenish to yellow-brown thoracic lobes, and usually only the antennae and siphunculi noticeably darker than in the aptera, Apterae 1.7-3.6mm, alatae 1.7-3.4mm. Dorsal abdomen without any clear intersegmental markings, Terminal process more than 2.5 times longer than base of last antennal segment, Siphunculi tapering, cylindrical or clavate, not coarsely imbricated. Small, medium rather large aphids, Inner faces of antennal tubercles clearly divergent, Siphunculi long, with a subapical zone of polygonal reticulation, Cauda long, 0.14-0.20 of body length (Singh and Boiteau, 1986, Joshi *et al.*, 2013).

12. *Brevicoryne brassicae* (Linnaeus)

Apterae medium-sized, greyish-green or dull green, with dark head and dark dorsal thoracic and abdominal markings, Body covered with greyish-white mealy wax which is also secreted on to the surface of the plant and extends throughout the colony, Alatae with dark head and thorax and black transverse bars on dorsal abdomen. Antennae 0.6-0.65 times of the body length, Siphunculi barrel shaped, dark, Siphunculi and cauda usually equal in length, sometimes cauda little

longer, with 6-10 hairs, Processus terminalis 3 to 3.2 times longer than the base of last antennal segment (Joshi *et al.*, 2013).

13. *Lypaphis erysimi* (Kaltenbach)

Apterae are small to medium-sized, yellowish green, grey green, or olive green, with a white wax bloom; in humid conditions often more densely coated with wax, Alatae have a dusky green abdomen with conspicuous dark lateral sclerites, and dusky wing veins, Sometimes in large numbers on the undersides of leaves, which may curl and turn yellow, or in inflorescences, Apterae 1.4-2.4 mm, alatae 1.4-2.2 mm, Presence of equidistant pleural oily patches on abdominal segment 1-4 and spinal bigger patches on segment 5-7, bases of siphunculi with oily patch, makes it easy to identify. Antennal tubercles small to moderately developed, but the median tubercle always well developed, Antennae 0.65 times as long as body, Processus terminalis almost double in length of base of last antennal segment, Ultimate rostral segment shorter than ht2, Siphunculi 0.12 times as long as body length and almost equal to or little longer than cauda, Cauda with four hairs (Joshi *et al.*, 2013).

CONCLUSIONS

Thirteen aphid species were found associated with potato crop in different parts of India namely *Myzus persicae*, *Aphis gossypii*, *Aphis fabae*, *Aphis spiraeicola*, *Aphis nerii*, *Hydaphis coriandri*, *Rhopalosiphum rufiabdominalis*, *Macrosiphum euphorbiae*, *Brevicoryna brassicae*, *Lypaphis erysimi*, *Rhopalosiphoninus latysiphon*, *Aulacorthum solani* and *Brachycaudus helichrysi* using morphological and molecular approaches. The relative importance of the various aphid species as vectors of potato viruses needs to be worked out and management thresholds might need adjustment accordingly for healthy seed potato production.

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