Indian Journal of Agricultural Sciences **88** (8): 1258–62, August 2018/Article

Role of alternate host plants in the transmission of apical leaf curl disease of potato caused by *tomato leaf curl New Delhi virus* – potato (ToLCNDV-pot.) in Northern India

R P PANT[1], ANUJ BHATNAGAR[2] AND MURARI LAL[3]

ICAR-Central Potato Research Institute, Regional Station, Modipuram, Meerut, Uttar Pradesh 250 110

Received: 25 August 2017; Accepted: 09 May 2018

ABSTRACT

Whitefly (*Bemisia tabaci* Gennadius) is a plant sap-sucking insect and transmits begomovirus, *tomato leaf curl New Delhi virus*-potato (ToLCNDV-potato) causing potato apical leaf curl disease has been posing serious threat to potato production in Northern India. Therefore, a field survey was conducted in Northern India during 2013-14 and 2014-15 potato offseason to identify the host plants, activity of whitefly on these plants with confirmation of ToLCNDV-potato through PCR. The presence of whitefly and PCR results revealed that cultivable host plants *Abelmoschus esculentus*, *Capsicum annum*, *Dahlia pinnata, Luffa cylindrica, Solanum melongena, Tagetes erecta, Vigna radiata* and non-cultivable host plants *Phyllanthus niruri, Trifolium repens, Acalypha indica* and *Commelina benghalensis* acquired ToLCNDV-potato in due course of time, however some of the alternate host plants were found negative, while whitefly collected from these plant showed positive reaction or vice versa. The findings of this study would help in studying the movement of whitefly and survival of ToLCNDV-potato on alternate host plants for better management of this disease in potato. As a precaution, a care should be taken to remove these plants in the vicinity of potato breeder seed crop in whitefly endemic area for the effective management of apical leaf curl disease of potato.

Key words: Host plants, PCR, Potato, ToLCNDV-potato, Whitefly

Whitefly (*Bemisia tabaci* Gennadius) is a plant sap-sucking insect in the family Aleyrodidae (Order: Hemiptera), commonly known as sweet potato whitefly (Bellows *et al.* 1994). This insect damages the crop by extracting large quantities of phloem sap, which can result in yield reduction (Muniyappa 1980). The natural infestation of white fly has been reported to reduce the plant growth and yield (Kumar and Jain 1992) from 12 to 45% and from 39.5 to 41.2%, respectively. Whiteflies are well known for their capacity to transmit 28 plant viruses of many crops (Galvez *et al*. 1989). This pest is reported to be an efficient vector of Gemini virus (Shrestha *et al.* 1997). Garg *et al.* (2001) reported that whitefly act as a vector which transmits potato apical leaf curl begomo virus which causes potato apical leaf curl disease. The virus ToLCNDV –potato, belongs to the family Geminiviridae and genus Begomovirus. The nucleotide sequencing of the virus indicates that the virus is a strain of tomato apical leaf curl New Delhi virus (ToLCNDV – potato). Lakra (2003) observed that due to apical leaf curl disease in early sown potato (1st week of October) severe yield losses were recorded at Hisar.

The preference of whitefly to its numerous hosts is not uniform and some hosts must be preferred to others for feeding and breeding (Mohanty and Basu 1991). The favored hosts reported are, potato, toria (Pruthi 1969), tomato (Singh *et al.* 1999), cotton (Butter and Kular 1999), cabbage, sarson, okra, pulses (Atwal 1986), cauliflower, lady's finger, cucurbits (Pruthi 1969), soybean (Chaudhary *et al.* 1981), melon (Geraud *et al.* 1991), tobacco (Aoki *et al*. 1995), brinjal (Kumar and Jain 1992), mungbean (Patel and Srivastava 1990), rice (Alam 1989), chickpea, cowpea, niger, groundnut, sesame (Moore *et al.* 2004) and some common weeds (Coudriet *et al.* 1986).

Large number of host plants of whitefly has been reported from different regions. However, information of cultivable and non-cultivable host plants with presence of whitefly and reservoir of ToLCNDV-potato is not available for North-Western India. A field survey therefore was conducted in Northern India during potato offseason to identify the host plants, activity of whitefly on these plants with confirmation of ToLCNDV-potato through PCR to understand the movement of whitefly and source of ToLCNDV-potato on alternate host plants for better management of this disease in potato.

[1]Principal Scientist (e mail: rajendrappant@gmail. Com), Division of Plant Pathology, ICAR-IARI, New Delhi. [2]Principal Scientist (e mail: dr.anujbhatnagar@gmail.com), ICAR-CPRS Modipuram, Meerurt, Entomology, [3]Assisrtanrt chief Technical Officer (e mail:muraricpri@gmail.com), ICAR-CPRS Modipuram, Meerut.

MATERIALS AND METHODS

A weekly field survey was carried out after the harvest of potato in the months of April to September (Offseason of potato) in Northern India during 2013-14 and 2014-15 with the objective to record the presence of whitefly and reservoir of ToLCNDV-potato in 20 cultivable and 10 non-cultivable host plants widely present in and around Central Potato Research Station, Modipuram, UP. The incidence of ToLCNDV was also detected in cultivable and non-cultivable host plants using PCR. The period of infestation and peak period of whitefly activity was recorded at weekly intervals and 10 numbers of adult and nymphs were used for detection of ToLCNDV. The presence of whitefly population of both nymphs and adults were observed during early morning hours on upper, middle and lower leaves from 5 selected and tagged plants.

Each leaf sample (100 mg) was washed thoroughly and dried and ground in 700 µl of CTAB buffer (2.0 % (w/v) CTAB, 20 mM EDTA (pH-8.0), 1.4 mM NaCl, 100 mM Tris-Cl (pH-8.0) and 0.2 % (v/v) 2-Mercaptoethanol). The extract was incubated at 65^0C for 30 min and centrifuged at 12,000 rpm for 1 min. 700 µl of chloroform: Isoamy alcohol (24:1 µl) was added to the supernatant and centrifuged at 12000 rpm for 20 min at room temperature. The aqueous phase was collected and added 2/3rd vol. of isopropanol and mixed gently by inversion and centrifuged at 12,000 rpm for 10 min to spin down the DNA pellet. The pellet was washed with 70% alcohol by spinning it at 10000 rpm for 10 min at 4^0C. The pellet was dried and dissolved in 30 µl of sterile water or TE buffer. Finally, 2.0 µl of RNAse (10.0 mg/ml) was added to the purified DNA and incubated at 37°C for 30 min. The concentration and quality of DNA was checked by Nanodrop -2000 Spectrophotometer.

PCR was performed using virus specific primers designed from CP sequences available in GenBank. The forward and reverse primers designed were LCVCPF1-5' AAAGTCCATGTGTGTTAGTGATGTTACC-3' and LCVCPR1-5' TAGAAATAGATCCGGATTTTCAAAGTA-3' (Jeevalatha *et al.* 2013). The coat protein gene of total isolated DNA of each sample was amplified. The PCR amplification was conducted in 20.0 µl of reaction volume containing 2.0 µl of template DNA (50 ng of total DNA), 2.0 µl of 10 x PCR buffer (100 mM Tris-HCl pH-9.0, 500 mM KCl, 15 mM MgCl2), 0.5 µl of 2 mM dNTPs, 0.5 µl of 10 µM forward and reverse primers respectively and 1.0 µl of (1.0 U/ µl) Red *Taq* DNA polymerase and 14.0 µl of sterile double distilled water. The thermal cycler was programmed for initial denaturation at 94^0 C for 5 min followed by 35 cycles at 94° C for 1 min, 62 °C for 1 min and 72 °C for 1 min and final extension of 10 min at 72°C. The amplified product was subjected to electrophoresis in 1% Agarose gel prepared in 1 × TAE (0.04M M Tris-acetate, 1mM EDTA, pH-8.0) buffer staining with (0.5 mg/ml) Ethidium bromide. Electrophoresis was carried out at 90 volt for 1-2 h. An aliquot (500 ng) of 1 Kb DNA ladder is used as molecular weight marker. The gel was visualized in UV trans-illuminator in gel documentation unit (Syngene G: Box) and scanned.

RESULTS AND DISCUSSION

The whitefly incidence on alternate cultivable and non-cultivable (weeds) host plants were presented in Table 1 during offseason of potato. The whitefly incidence data on different hosts revealed that the whitefly has moved from one plant to other suitable host plant before hovering early potato and transmit the ToLCNDV on these hosts. These plants acted as a reservoir for the survival of ToLCNDV during offseason of potato. Whiteflies were recorded on *Abelmoschus esculentus*, *Capsicum annum*, *Dahlia pinnata*, *Duranta repens, Coccinia grandis*, *Gossypium, Luffa cylindrica, Ocimum sanctum, Solanum melongena, Tagetes erecta, Vigna mungo, V. radiata* and *V. unguiculata* from April to December during both the years depending upon availability of suitable host plant. A high population of whiteflies (more than 100/ leaf) was also recorded on *O. sanctum* and *V. mungo* during June and August. No incidence of whitefly was recorded on *Chrysanthemum, Digenea arvensis, Momordica charantia, Morus alba*, *Brassica juncea, Carica papaya* and *Rosa alba.* Among the non-cultivable host plants *Phyllanthus niruri* and *Trifolium repens* were heavily infected by whitefly during April to August. *Acalypha indica*, *Alternenthera pungens* and *Commelina benghalensis* were also found as the host of whitefly during off season of potato. Later, whiteflies were migrated to early planted potato in the first week of October.

The viruliferous nature of whiteflies and leaves of host plants with respect to ToLCNDV-potato was determined using virus specific primers designed from CP sequences available in GenBank along with internal control. Leaf samples of fourteen cultivable and ten non cultivable host plants were tested for the presence of ToLCNDV-potato through PCR. The results of PCR reaction were presented in Table 2, Fig 1. The leaves of *A. esculentus*, *C. annum, C. grandis* , *D. pinnata*, *L. cylindrica, C. papaya, S. melongena, T. erecta* and *V. radiata* showed positive reaction for the presence of ToLCNDV-potato through PCR. Ten non cultivable plants were tested for the presence of virus; only six plants were found positive against ToLCNDV-potato while three were found free from the virus. Similarly, whitefly samples collected from these plants were further evaluated for the presence of ToLCNDV-potato through PCR. Results presented in Table 2 and Fig 1 revealed that whitefly collected from cultivable host plants like *A. esculentus*, *C. annum*, *D. pinnata* , *D.repens, L. cylindrica, S. melongena, T. erecta, V. radiata* and *V. unguiculata* found positive for ToLCNDV-potato. However, whitefly collected from other cultivable host plants were found free from ToLCNDV-potato.

The presence of whitefly and PCR results revealed that cultivable host plants *A. esculentus*, *C. annum*, *D. pinnata, L. cylindrica, S. melongena, T. erecta, V. radiata* and non-cultivable host plants *P. niruri, T. repens, A. indica* L and *C. benghalensis* acquired ToLCNDV-potato in due course

Table 1 List of alternate host plants (cultivable and non-cultivable) of tomato leaf curl New Delhi virus-pot. (*ToLCNDV*)-pot. and period of whitefly infestation

Common name	Scientific name	Plant family	Period of infestation		Peak period
			From	To	
Okra	*Abelmoschus esculentus* (L)	Malvaceae	May	September	June
Chilli	*Capsicum annum*	Solanaceae	May	September	June
Chrysanthemum	*Chrysanthemum*	Asteraceae	-	-	-
Kundru	*Coccinia grandis*	Cucurbitaceae	May	September	June
Cotton	*Gossypium*	Malvaceae	April	October	September
Dahlia	*Dahlia pinnata*	Asteraceae	May	September	June
Mint	*Digenea arvensis*	Primulaceae	-	-	-
Golden duranta	*Duranta repens*	Verbenaceae	May	September	June
Torai	*Luffa cylindrica*	Cucurbitaceae	April	October	June
Bitter gourd	*Memordica charantia*	Cucurbitaceae	-	-	-
Mulberry	*Morus alba*	*Moraceae*	-	-	-
Mustard	*Brassica juncea*	Cruciferae	-	-	-
Tulsi	*Ocimum sanctum*	Lamiaceae	April	October	July/August
Papaya	*Carica papaya*	Caricaceae	-	-	-
Rose	*Rosa alba*	Rosaceae	-	-	-
Brinjal	*Solanum melongena*	Solanaceae	April	October	July/August
Marigold	*Tagetes erecta*	Compositae	July	December	September
Blackgram	*Vigna mungo*	Legumacae	June	August	July/ August
Mungbean	*Vigna radiate*	Legumacae	May	June	June
Cowpea	*Vigna unguiculata*	Legumacae	May	June	June
Alternative non-cultivated host plants and period of whitefly activity					
Bathua	*Chenopodium amranticolor*	Amaranthaceae	October	March	October
Indian Nettle	*Acalypha indica* L	Euphorbiaceae	September	March	October
Chirchita (Latjira)	*Achyranthes aspera*	Amaranthaceae	September	March	October
Khaki weed	*Alternenthera pungens*	Amaranthaceae	September	March	October
Bengal dayflower	*Commelina benghalensis*	Commelinaceae.	September	March	October
Asthma weed	*Euphorbia hirta*	Euphorbiaceae	April	October	July/August
Bhoomi amala	*Phyllanthus niruri*	Euphorbiaceae	April	August	July
Makoy	*Solanum nigrum*	Solanaceae	-	-	-
Dhaman grass weed	*Tridax procumbens*	Asteraceae	-	-	-
Red clover	*Trifolium repens*	Fabaceae	April	August	July

of time. However, some of the alternate host plants showed negative reaction, while whitefly collected from these plant showed positive reaction or vice versa. It showed some of the host plants are susceptible and quickly acquired virus under the favorable environmental condition. The virus acquired by these viruliferus whitefly further spread the virus in early sown potato cultivars. The above results revealed that ToLCNDV-potato multiplied throughout the year and transmitted by whitefly from one host plant to another host plant. The buildup of whitefly on host plants was also influenced by weather factors (Bhatnagar 2007). Presently, the whitefly has become a severe threat to potato crop by sucking the sap directly from the tender parts of potato and transmitting ToLCNDV (Bhatnagar 2007 and Lakra 2003). The yield loss in potato caused by whitefly is mainly due to transmission of potato apical leaf curl virus (Bhatnagar 2007).

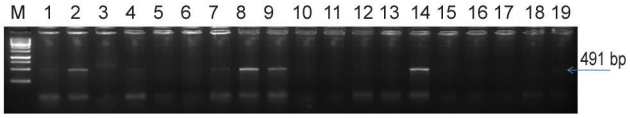
Our finding further supported by earlier finding of the

Table 2 PCR results showing ToLCNDV-pot. in alternate host plants and whitefly

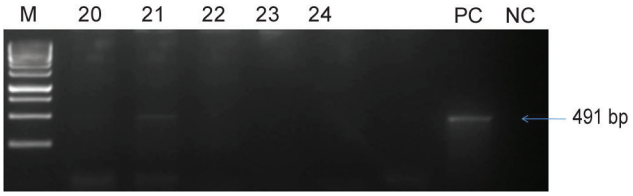
Name of host plants	PCR results of *ToLCNDV*-potato of	
	Whitefly collected from plants	Leaves collected from host plants
Cultivated plants		
Abelmoschus esculentus (L)	+	+
Capsicum annuum	+	+
Chrysanthemum	-	ND
Coccinia grandis	ND	+
Gossypium	-	-
Dahlia pinnata	+	+
Digenea arvensis	-	ND
Duranta repens	+	ND
Luffa cylindrica	+	+
Memordica charantia	ND	-
Morus alba	-	-
Brassica juncea	-	ND
Ocimum sanctum	-	ND
Carica papaya	ND	+
Rosa alba	ND	-
Solanum melongena	+	+
Tagetes erecta	+	+
Vigna mungo	ND	-
Vigna radiate	+	+
Vigna unguiculata	+	ND
Non-cultivated plants		
Chenopodium amranticolor	-	+
Acalypha indica	+	+
Achyranthes aspera	-	-
Alternenthera pungens	+	ND
Commelina benghalensis	+	+
Euphorbia hirta	-	+
Phyllanthus niruri	+	+
Solanum nigrum	-	-

- Negative, + positive and ND- not done

favored hosts plants as reported on potato, toria, (Pruthi 1969), tomato (Singh *et al.* 1999), cotton (Butter and Kular 1999), cabbage, sarson, okra, pulses (Atwal 1986), cauliflower, lady's finger, cucurbits (Pruthi 1969), soybean (Chaudhary *et al.*1981), melon (Geraud *et al.* 1991), tobacco (Aoki *et al*. 1995), brinjal (Kumar and Jain 1992), mungbean (Patel and Srivastava 1990), rice (Alam 1989), chickpea, cowpea, niger, groundnut, sesame (Moore *et al.* 2004) and some common weeds (Coudriet *et al.* 1986). Singh *et al.* (1994) observed a greater buildup of the whitefly population on cotton during October in Punjab. Singh and Jaglan (2001) in their studies on brinjal reported that *Bemisia tabaci* though was present throughout the year,



Luffa cylindrica 2. *Duranta repens* 3. *Vigna radiate* 4. *Dahlia pinnata* 5. *Morus alba* 6. *Phyllanthus niruri* 7. *Tagetes erecta* 8. *Vigna unguiculata* 9. *Acalypha indica* 10. *Commelina benghalensis* 11. *Euphorbia hirta* 12. *Tridax procumbens* 13. *Capsicum annum* 14. *Abelmoschus esculentus* 15. *Ocimum sanctum* 16. *Chrysanthemum* sp.17. *Solanum melongena* 18. *Gossypium* sp.19 *Solanum nigrum*



20. *Achyranthes aspera* 21. *Alternenthera pungens* 22. *Digenea arvensis* 23. *Brassica juncea* 24. *Chenopodium amranticolor* , PC= Positive control , NC= Negative control

Fig 1 PCR detection of ToLCNDV-potato from whitefly collected from different host plants

but its activity increased during *kharif* season from June to September when humidity and temperature were quite high. The peak period of whitefly in western Uttar Pradesh was from October-November (Raj 2003). He observed an increase in whitefly population rapidly from July to September. After the defoliation of cotton was initiated in September, whiteflies migrated to melons and then to lettuce. Muthukumar and Kalyanasundaram (2003) observed that *Bemisia tabaci* appeared from the first week of April after transplanting and persisted throughout the season.

It is concluded that the PCR reaction confirmed the presence of ToLCNDV-potato in a wide range cultivable and non-cultivable host plants are working as source of virus and subsequently transmission of virus in early potato crop from these viruliferus whitefly under the favorable environmental condition. Therefore, removal of these alternate host plants growing near to potato breeder seed crop in whitefly endemic area is strictly needed for the effective management of apical leaf curl disease in potato. Although the early planted crop is more prone to the attack by whitefly, the main crop may also suffer to some extent. On the other hand, the pest- spectrum is likely to change drastically in late sown crop.

ACKNOWLEDGEMENT

Authors are highly thankful to Director, CPRI, Shimla and Joint Director, CPRI-Regional Station, Modipuram, Uttar Pradesh for providing necessary facilities to conduct this study.

REFERENCES

Alam M S. 1989. Whitefly (Hemiptera: Aleyrodidae) – a potential pest of rice in West Africa. *International Rice Research Newsletter (Philippines)* **14** (3): 38–9.

Aoki K, Yamada H and Simohata T. 1995. Epidemiology and control of *Bemisia tabaci* Gennadius (*Nicotiana tabacum*) in Gifu Pref. (Japan). *Bulletin of the Gifu Agricultural Research Center* 8: 23–36.

Atwal A S. 1986. Polyphagous pests. (*In*) *Agricultural Pests of India and South East Asia*, II edition, pp 346–66. Kalyani Publishers, New Delhi.

Bellows T S, Perring T M, Gill R J and Headrick D H. 1994. Description of a species *Bemisia* (Homoptera : Aleyrodidae). *Annals of the Entomological Society of America* **87**: 195–206.

Bhatnagar A. 2007. Incidence and succession of thrips, leafhoppers and whitefly in combination of planting dates and potato varieties. *Annals of Plants Protection Sciences* **15**(1): 101–5.

Butter N S and Kular J S. 1999. Resurgence of whitefly in cotton and its management, *Indian J. Ent.* **61**(1): 85–90.

Chaudhary R R P, Bhattacharya A K and Rathore R R S. 1981. Use of systemic insecticides for the control of stem fly *Melanagromyza sojae* (Zehnt.) and whitefly, *Bemisia tabaci* Genn. *Indian J. Ent.* **43**(2): 223–5.

Coudriet D L, Meyerdirk D E, Prabhakar N and Kishaba A N. 1986. Bionomics of sweet potato whitefly (Homoptera: Aleyrodidae) on weed hosts in the Imperial Valley, California. *Environmental Entomology* **15**(6): 1179–83.

Galvez G E. 1989. Whitefly-transmitted viruses. *Bean Production Problems in the Tropics, pp 379–406.* Schwartz H F and Pastor-Corrales M A. (*Eds). Centro Internacional de Agricultura Tropical,* Cali (Colombia).

Garg I D, Khurana S M P, Kumar S and Lakra B S. 2001. Association of geminivirus with potato apical leaf curl in India, and its immune, electron microscopic detection. *J. Indian Potato Assoc* **28**: 227–32.

Geraud F, Arcila S and Pena M. 1991. Population dynamics of the tobacco whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), on melon, in El Cebollal, Municipality Miranda, state Falcon, Venezuela. *Revista de la Facultad de Agronomia* **8**(4): 228.

Kumar Sushil and Jain Padam . 1992. Comparative bio-efficacy of various insecticides against whitefly *Bemisia tabaci* Genn. on brinjal, *Indian J. Ent.* **54**(2): 124–31.

Mohanty A K and Basu A N. 1991. Relative preference of *Bemisia tabaci* (Genn.) fos some of its common host plants during different seasons. *Journal of Entomological Research* **15**(1): 70–1.

Lakra, B S. 2003. Effect of date of planting on whitefly population, leaf curls incidence and yield of potato cultivars. *J. Indian Potato Assoc.* **30**: 115–6.

Moore A D, Sequeira R V and Woodger T A. 2004. Susceptibility of crop plants to *Bemisia tabaci* (Gennadius) B-biotype (Hemiptera: Aleyrodidae) in Central Queensland, Australia. *Australian Entomologist* **31**(2): 69–74.

Muthukumar M and Kalyanasundaram M. 2003. Influence of abiotic factors on the incidence of major insect pests in brinjal (*Solanum melongena* L.). *South Indian Horticulture* **51**(1/6): 214–8.

Jeevalatha A, Kaundal P, Venkatasalam E P, Chakrabarti S K and Singh B P. 2013. A robust technique for the detection of tomato leaf curl New Delhi virus-potato in ultra low virus titre. *Potato J.* **41**:76–80.

Muniyappa V. 1980. *Whiteflies-On Vectors of Plant Pathogens,* pp 39–85. Harris K F and Maramorosch K (Eds). *Academic Press,* New York.

Patel M P and Srivastava K P. 1990. Field screening of some high yielding genotypes of mungbean, *Vigna radiata* (Linnaeus) Wilczek to whitefly, *Bemisia tabaci* (Gennadius) and yellow mosaic virus (YMV) *Indian J. Ent.* **52**(4): 547–51.

Pruthi H S. 1969. The more injurious pests of important crops. (*In*) *Textbook on Agricultural Entomology*, pp 576–681. Kachroo P (Ed). ICAR, New Delhi.

Singh D and Jaglan R S. 2001. Efficacy of seedling root dip method of insecticides against whitefly (*Bemisia tabaci* Genn.) on brinjal *J. Ent. Res.* **25**(4): 293–8.

Singh J, Sohi A S, Mann H S and Kapur S P. 1994. Studies on whitefly, *Bemisia tabaci* (Genn.) transmitted cotton leaf curl disease in Punjab *Journal of Insect Science* 7(2): 194–8.

Singh B P, Verma K D and Chandla V K. 1999. Integrated disease and pest management package for potato, *Indian Farming* **49**(9): 58–60 / 63.

Raj B T. 2003. Studies on potato pests in western Gangetic plains. *J. Exp. Zool.* **6**(2): 397–401.

Shrestha K, Shrestha S K, Timila R D and Joshi S. 1997. Tomato leaf curls disease: a white fly transmitted gemini virus of tomato in Nepal. *Nepalese J. Agri.* **18-28**: 107–12.