Non-tomato natural hosts of tomato infecting begomoviruses in north-western India

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SUMMARY: Four whitefly-transmitted geminiviruses (WTGs), belonging to the genus *Begomovirus*, cause leaf curl disease of tomato (TomLCD) in India. These viruses have a wide host range. To identify the natural non-tomato hosts and distribution of these viruses, samples from eight weed and ornamental plant species, showing symptoms typical of those induced by WTGs were collected from areas around tomato fields in Delhi, Haryana, Punjab and Rajasthan. The samples collected from Delhi were tested by (a) transmission by whitefly (*Bemisia tabaci*) to tomato, (b) nucleic acid spot hybridization to detect viral genomic components, and (c) polymerase chain reaction to detect the viruses known to cause TomLCD in India. Whereas, the samples collected from Haryana, Punjab and Rajasthan were tested only by PCR. In all the eight plant species tested from Delhi, DNA A and DNA β were detected, but DNA B was detected only in *Solanum nigrum* by NASH with probes of DNA A, DNA B and DNA β of *Tomato leaf curl New Delhi virus* (ToLCNDV). By PCR, using primers designed from the conserved coat protein (CP) and intergenic (IR) regions of DNA A of the four WTGs causing TomLCD in India, amplification was obtained from six of the eight plant species tested from Delhi. The WTGs from these six plant species were also transmitted to tomato cv. Pusa Ruby by whitefly; the infected tomato plants developed leaf curl symptoms with minor variations. Although WTGs were transmitted from six plant species collected from Delhi, the WTGs known to cause TomLCD in India were detected only in two plant species by PCR with virus species specific primers; ToLCNDV was detected in *S. nigrum* and *Tomato leaf curl Gujarat virus* (ToLCGV) was detected in *Datura metel*. ToLCNDV and ToLCGV were also detected by PCR in samples of *S. nigrum* collected from Punjab, and wild sunflower from Rajasthan, respectively. This is the first report of molecular detection of tomato infecting begomoviruses in *D. metel, Eclipta alba, Tagetes erecta, Phyllanthus niruri, S. nigrum* and wild sunflower, and first report of *Begomovirus* infection in *Tabernaemontana divaricata*. ToLCNDV has been detected for the first time in *S. nigrum* and cluster bean, and ToLCGV in *D. metel* and wild sunflower. The other two WTGs - *Tomato leaf curl Bangalore virus* (ToLCBV) and *Tomato leaf curl Karnataka virus* - causing TomLCD in southern India were not detected in any of the plant species tested in this study indicating that these two viruses do not occur commonly in Delhi, Haryana, Punjab and Rajasthan. These findings indicate *D. metel, S. nigrum* and wild sunflower may play an important role in the perpetuation of ToLCNDV and ToLCGV in north-western India. The present study also shows occurrence of so far uncharacterized WTGs in weeds and ornamental plants, which may be causing TomLCD in north-western India.

Key words: *Datura metel, Eclipta alba, Phyllanthus niruri, Solanum nigrum, Tabernaemontana divaricata, Tagetes erecta,* wild sunflower, Begomovirus, whitefly transmission, NASH, PCR

INTRODUCTION

Leaf curl disease of tomato (TomLCD) is known to affect tomato cultivation in north-western India for nearly 60 years (Vasudeva and Samraj, 1948). The disease, caused by whitefly transmitted geminiviruses (WTGs) belonging to the genus *Begomovirus,* has been reported from most of the tomato growing areas of the world, causing yield losses of up to 100 per cent depending upon the stage of infection (Butter and Rataul, 1981; Kalloo, 1996; Sastry and Singh, 1973; Saikia and Muniyappa, 1986; Nainar and Pappiah, 1999; Varma and Malathi, 2003). The begomoviruses are characterized by single stranded circular DNA packed in quasi-icosohedral particles. The genome of begomoviruses is either bipartite, designated as DNA A and DNA B (each of about 2.7 Kb), or monopartite designated DNA A (about 2.7 Kb) (Varma and Malathi, 2003; Guterrez *et al.,* 2004; Fauquet and Stanley, 2005). In addition to DNA A and DNA B, satellite-like DNA β molecules have also been found associated with some of the monopartite (Saunders *et al.,* 2000; Briddon *et al.,* 2001; Zhou *et al.,* 2003; Jose and Usha, 2003; Radhakrishnan *et al.,* 2003; Bull *et al.,* 2004) and bipartite (Sivalingam *et al.,* 2004) begomoviruses. The number of begomoviruses causing diseases in tomato is increasing at a fast rate. Until 2003, 52 begomoviruses were reported to cause diseases in tomato in various parts of the world (Varma and Malathi, 2003), and by 2005 the number has swelled to 71 (Fauquet and Stanley, 2005). Many of these cause TomLCD, and are commonly known by the generic names like 'tomato leaf curl virus' and 'tomato yellow leaf curl virus'. These

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viruses have a wide host range and infect a large number of dicotyledonous plants belonging to different families.

In India, *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Bangalore virus* (ToLCBV), *Tomato leaf curl Karnataka virus* (ToLCKV) and *Tomato leaf curl Gujarat virus* (ToLCGV) have been identified to cause TomLCD. Two of these, ToLCNDV and ToLCGV, have bipartite genome (Padidam *et al.*, 1995; Srivastava *et al.*, 1995; Tripathi and Varma, 2003; Chakraborty *et al.*, 2003), and ToLCBV and ToLCKV have monopartite genome (Hong and Harrison, 1995; Muniyappa *et al.*, 2000; Kirthi *et al.*, 2002; Chatchawankanphanich and Maxwell, 2002). Various crop plants and weeds are known to be hosts of theses viruses (Vasudeva and Samraj, 1948; Sastry *et al.*, 1978; Rataul and Brar, 1989; Nariani, 1968; Reddy and Yaraguntaiah, 1982; Saikia and Muniyappa, 1986).

In many parts of India tomato is cultivated throughout the year. This round the year cultivation helps in the perpetuation of the viruses and their transmission by the vectors to successive tomato crops (Tripathi and Varma, 2002). These viruses can also survive in non-tomato hosts (weeds and other crops) during the off-season. Some of these alternative hosts are permanent reservoirs of the viruses. For developing suitable management strategies, it is essential to identify the alternative hosts, which harbour these viruses and help their survival. This information can be utilized in adopting cropping patterns/ systems and cultural practices to reduce the availability of virus source(s) adjoining the tomato crops. Earlier studies (Sastry *et al.*, 1978; Gupta *et al.*, 2001) have indicated the role of alternative hosts in the spread of TomLCD, but only limited information is available on the alternative hosts of specific TomLCD associated virus species; ToLCNDV has been found to cause serious diseases in *Luffa cylindrica* (Sohrab *et al.*, 2003) and potato (Usharani *et al.*, 2004). In this study, the altenative hosts of TomLCD associated begomoviruses have been identified by molecular diagnosis and transmission by vector whitefly (*Bemisia tabaci*) to tomato.

MATERIALS AND METHODS

Collection of samples from naturally infected weeds and other plants

Weed, ornamental and crop plants showing virus-like symptoms were collected from areas around tomato fields in Delhi, Haryana, Punjab and Rajasthan.

Whitefly transmission

The stem ends of the branches of symptomatic plant samples collected from Delhi were kept immersed in water, to maintain turgidity of the leaves, before acquisition feeding by whitefly for transmission. About 50 non-viruliferous whiteflies, maintained on *Nicotiana tabacum* were allowed to feed on the symptomatic leaves for 24 h. After the acquisition access period (AAP), ten viruliferous whiteflies were transferred to each of the 10-15 day old healthy tomato cv. Pusa Ruby plants, and were given inoculation access period (IAP) of 24 h. After the IAP whiteflies were killed by spraying insecticide (Decis 2.8@0.05%) and the inoculated plants maintained in an insect-proof glasshouse for 30 days for symptom development.

DNA extraction

Total DNA was extracted from all the samples by CTAB method (Dellaporta *et al.*, 1983). For nucleic acid spot hybridization (NASH), DNA was extracted from 1 g leaf tissue and for PCR 100 mg of leaf tissue from each sample was used. The final DNA pellets were dissolved in sterile double distilled water.

Nucleic acid spot hybridization (NASH)

Approximately five µg of total DNA extracted from the plants to be tested was spotted on nylon membrane (NCM; Amersham Inc., USA). After spotting the membranes were dried and baked at 80°C for 2 h. Standard procedure of hybridization was followed (Sambrook *et al.*, 1989). For hybridization, specific probes were prepared from the clones of DNA A, DNA B and DNA β of isolate ToLCNDV-De (obtained from the Advanced Centre for Plant Virology, IARI, New Delhi). Probe to detect DNA A was prepared from the PCR amplified coat protein (CP) gene from DNA A clone, the probe of DNA β was prepared from the full length DNA β clone, and of DNA B from a fragment of ~ 500 bp obtained by the restriction of DNA B clone by *Kpn* I and *Pst* I. Radiolabelling of the probes was done by incorporating a-^{32}p dCTP by random priming method using Random Primer Labelling Kit (Genei Pvt. Ltd., Bangalore). The radio-labelled probes were individually added to pre-hybridization solution @ 0.5 x 10^6 dpm/ml and used for hybridization. Pre-hybridization and hybridization was done at 65°C for 4 and 18 h respectively. Hybridized blots were washed in 2 x SSC (0.3 M sodium chloride and 0.03 M sodium citrate, pH 7.4), containing 0.1% SDS thrice at 65°C for 15 min each time. Autoradiographs were prepared by exposing Kodak X-ray film for 24-48 h at -70° C.

Polymerase Chain Reaction (PCR)

Two sets of primers were designed, to amplify CP gene

and IR of tomato begomoviruses, on the basis of published nucleotide sequences of DNA A of tomato infecting begomoviruses in India (ToLCBV-[Ban4] (AF165098), ToLCBV-[Ban5] (AF295401), ToLCBV-[Kolar] (AF428255), ToLCNDV-[Svr] (U15015), ToLCNDV-[Luc] (Y16421), ToLCKV (U38239), ToLCGV-[Kello] (AY449999), ToLCGV-[Var] (AY190290) and ToLCGV-[Vadodara] (AY413671). The IR region primers were designed to (a) amplify all the begomoviruses known to cause TomLCD in India – this set is referred as 'common IR primers', and (b) species specfic primers to amplify the known *Begomovirus* species causing TomLCD in India. The following primers, used in this study were synthesized by the Genetix Biotech. Co., Germany.

CP gene primers

1. AV103F: 5′-ATGGTGAAGCGACCAGCAGAT-3′
2. AV104R: 5′-TTAATTTGTTACCGAATCTA-3′

Common IR primers

1. AV105F: 5′-CCCATCTCTCGTGAAGCTCTC-3′
2. AV106R: 5′-TCATTCAAAAGTGGATCCCAC-3′

ToLCNDV specific primers

1. AV105F: 5′-CCCATCTCTCGTGAAGCTCTC-3′
2. AV107R: 5′-AGTCTATTTAAAGGACCCC-3′

ToLCBV specific primers

1. AV105F: 5′-CCCATCTCTCGTGAAGCTCTC-3′
2. AV108R: 5′-TGGAGGGGACCATGACAG-3′

ToLCKV and ToLCBV specific primers

1. AV105F: 5′-CCCATCTCTCGTGAAGCTCTC-3′
2. AV109R: 5′-CGTTGTATGTAGACCAA-3′

ToLCGV and ToLCKV specific primers

1. AV105F: 5′-CCCATCTCTCGTGAAGCTCTC-3′
2. AV110R: 5′-AGTTAGTGCGTTGTGGGG-3′

Uniform PCR conditions were followed. The reaction mixture consisted of 5 µl 10 x PCR buffer with $NH_4(SO_4)_2$, 3 µl 25mM $MgCl_2$, 1 µl 10 mM dNTP, 1 µl 100 ng/µl each primer for CP (AV103F and AV104R) and IR (AV105F and AV106R), 1 unit *Taq* DNA polymerase (MBI Fermentas, USA) and ~ 500 ng of total DNA. Total reaction volume was made up to 50 µl using sterile double distilled water.

The following PCR programme was used in a thermocycler (Bio-metra); initial denaturation at 94ºC for 2 min, followed by 30 cycles of denaturation (94ºC for 1 min), annealing (55ºC for 2 min) and extension (72ºC for 3 min). At the end of the 30 cycles, a final extension step at 72ºC for 10 min was set and then the temperature was decreased to 4ºC until the reaction mixtures were removed. PCR products were analysed by electrophoresis in 1% agarose gel, stained with ethidium bromide (0.05 mg/ml) and photographed on a UV transilluminator.

RESULTS

Transmission by whitefly

The samples collected from eight weed and ornamental plants collected from Delhi showed varying symptoms (Table 1; Fig.1). The virus(es) from six of the eight samples was(were) successfully transmitted to tomato cv Pusa Ruby by whitefly inoculation. No transmission to tomato was obtained from *Coleus* sp. and *T. divaricata* (Table 1). The rate of transmission from other hosts varied from 25 to 40% and the time taken for symptom

Table 1. Whitefly transmission and detection of tomato infecting begomoviruses in non-tomato hosts

Name of the plant	Symptoms the weed hosts*	NASH test			PCR		Whitefly transmission to tomato$	Symptoms on tomato*	Symptom development (DAI)	No. tomato plants infected/ no. inoculated		Per cent transmission
		A	B	β	IR	CP				Expt. I	Expt. II	
Coleus sp.	LC	-	-	-	-	-	-	-	-	0/5	nd	-
Datura metel	DC	+	-	+	+	+	+	DC, Y	14	3/8	2/5	38.5
Eclipta alba	YV	+	-	+	+	+	+	LC	8	3/5	1/5	40.0
Tagetes erecta	UC, R	+	-	+	+	+	+	LC, Y	15	1/7	2/5	25.0
Parthenium hysterophorus	LC, SB	+	-	+	+	+	+	LC, M	15	2/7	2/5	33.3
Phyllanthus niruri	LC, YM	+	-	-	+	+	+	LC, SL	9	3/5	1/5	40.0
Solanum nigrum	LCr, YM	+	+	-	+	+	+	LC, Y	12	2/8	2/4	33.3
Tabernaemontana divaricata	LCr, M	+	-	-	-	+	-	-	-	0/7	nd	-

* LC– leaf curl; DC– downward curling; R– rolling; UC- upward curling; SB- stem bending; SL- small sized leaves; YV- yellow vein; LCr- leaf crinkle; M- mosaic; YM- yellow mosaic; Y-yellowing; nd- Not done

$ Ten whiteflies were used per plant with AAP of 24 hrs and IAP of 24 hrs

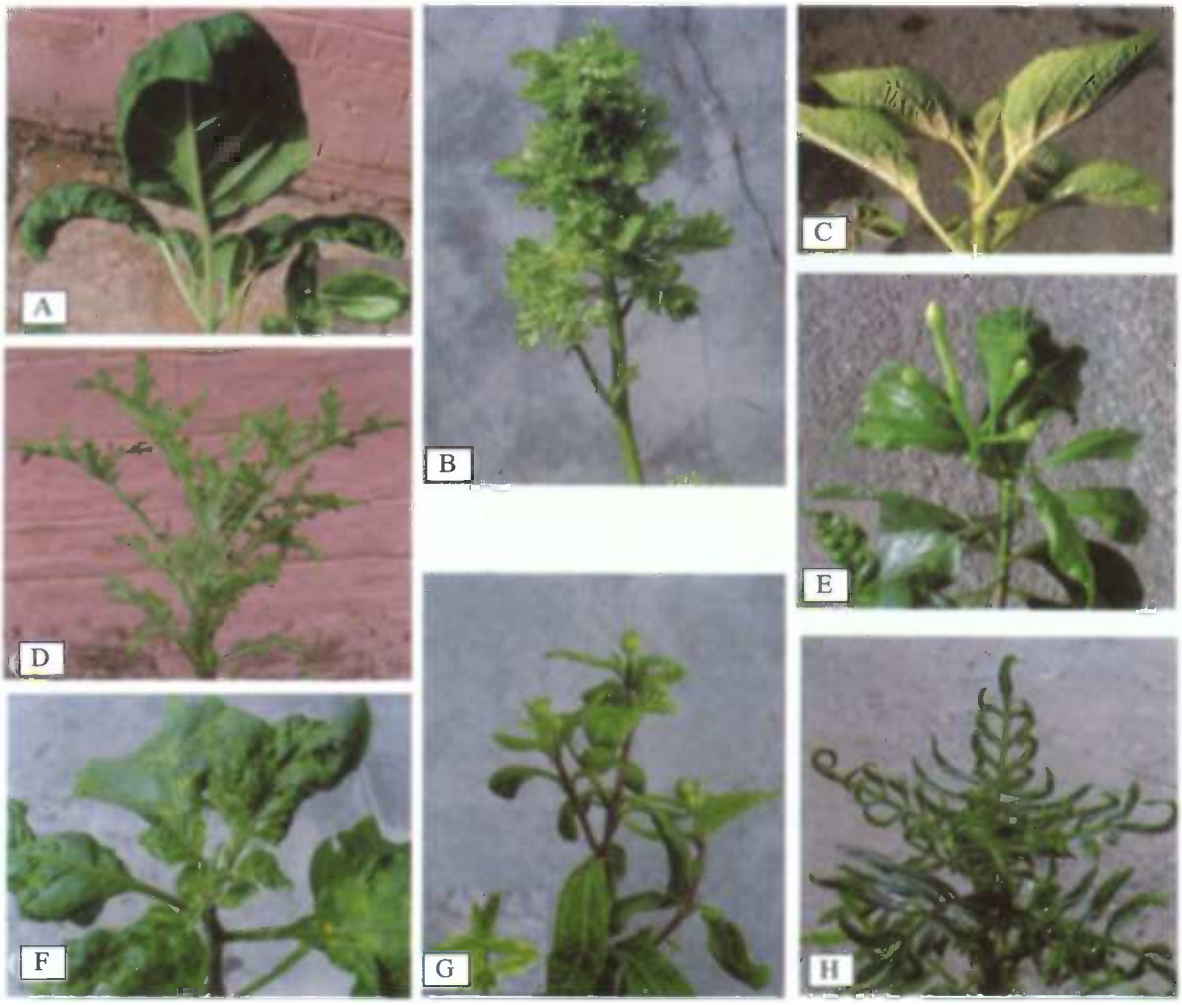

Fig. 1: Symptoms on weed hosts collected from Delhi (A) Datura metel, (B) Phyllanthus niruri, (C) Coleus sp., (D) Parthenium hysterophorus, (E) Tabernaemontana divaricata, (F) Solanum nigrum, (G) Eclipta alba and *(H) Tagetes erecta*

development varied from 8-15 days after inoculation (Table 1). The virus from *S. nigrum* induced symptoms similar to ToLCNDV infected tomato plants with curling and light greenish yellow pigmentation of the leaves (Fig. 2e). Plants infected with the virus from *P. hysterophorus* and *P. niruri* also showed typical leaf curl symptoms (Fig. 2d, f). Yellowing and curling was observed in tomato plants infected with the virus(es) from *D. metel* and *T. erecta* (Fig. 2a, c). Similarly, mild yellowing and curling symptoms developed in tomato infected with the virus from *E. alba* (Fig. 2b).

NASH

In NASH, seven of the eight samples tested from Delhi were positive for DNA A and four for DNA β. With DNA A probe, strong hybridization was obtained with *S. nigrum* compared to the other six hosts, which hybridized mildly. With DNA β probe strong hybridization was obtained with *D. metel* and *T. erecta* and mild hybridization with *E. alba* and *P. hysterophorus.* Very faint hybridization was obtained with the other four plant species, which were not considered positive for DNA β (Fig. 3). DNA B probe of ToLCNDV hybridized only with *S. nigrum* and not with any other plant tested.

PCR

PCR amplification was obtained, in seven of the eight plants tested from Delhi, with the CP gene primers AV103 and AV104, and six plants with the 'common IR primers' AV105F and AV106R (Table 1; Fig. 4 and 5). *T. divaricata*, however, gave limited amplification with the CP gene primers and no amplification with the common IR primers (Fig. 4 and 5). No amplification was obtained from the samples of *Coleus* sp., which also did not hybridize with DNA A and DNA β probes.

In addition to the eight samples collected from Delhi, three samples from Haryana, six from Punjab and 14 from Rajasthan were tested with the common IR and species specific primers (Table 2; Fig. 5). Three of the six samples from Punjab and two of the 14 samples from Rajasthan

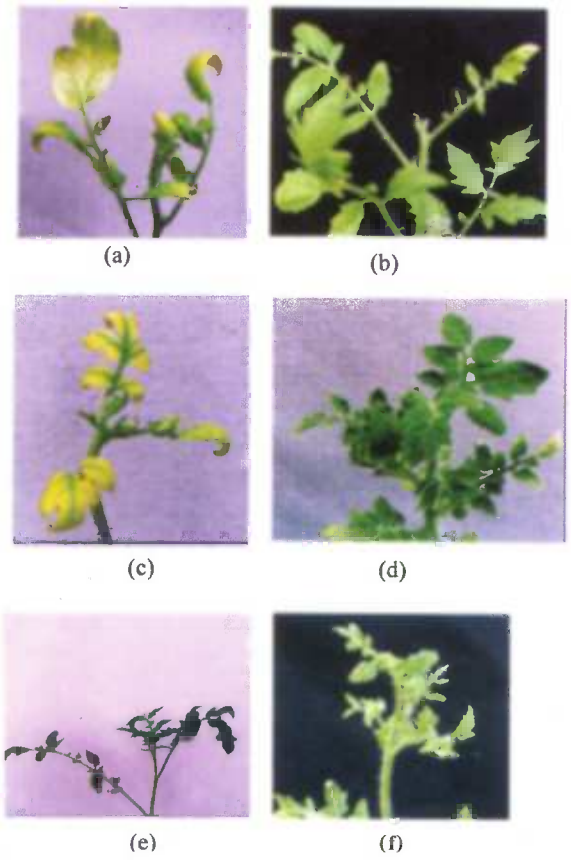

Fig. 2: Whitefly transmission from different hosts to tomato cv. Pusa Ruby showing different types of symptoms (a) Datura metel, *(b)* Eclipta alba, *(c)* Tagetes erecta, *(d)* Parthenium hysterophorus, *(e)* Solanum nigrum, *and (f)* Phyllanthus niruri

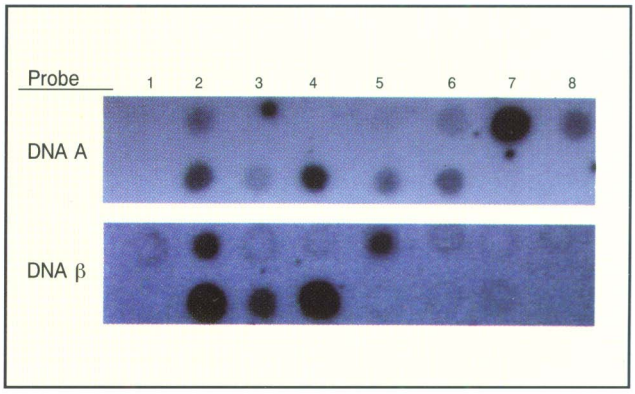

*Fig. 3: Detecion of DNA A and DNA β in weed and ornamental plants collected from Delhi by NASH using DNA A and DNA β as probe. 1-*Coleus sp.; *2-*Datura metel; *3-*Eclipta alba; *4-*Tagetes erecta; *5-*Parthenium hysterophorus; *6-*Phyllanthus niruri; *7-*Solanum nigrum; *8-*Tabernaemontana divaricata

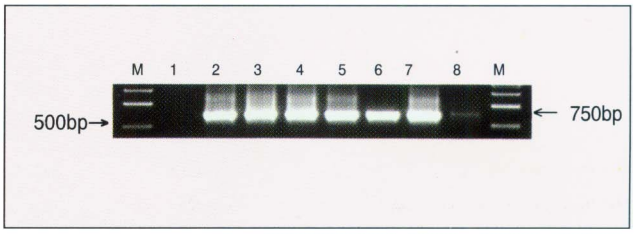

*Fig. 4: Agarose gel electrophoresis of PCR amplification of the coat protein gene from putative alternate hosts. Lane M-1Kb DNA ladder; 1-*Coleus *sp.; 2-*Datura metel; *3-*Eclipta alba; *4-*Tagetes erecta; *5-*Parthenium hysterophorus; *6-*Phyllanthus niruri; *7-*Solanum nigrum; *8-*Tabernaemontana divaricata

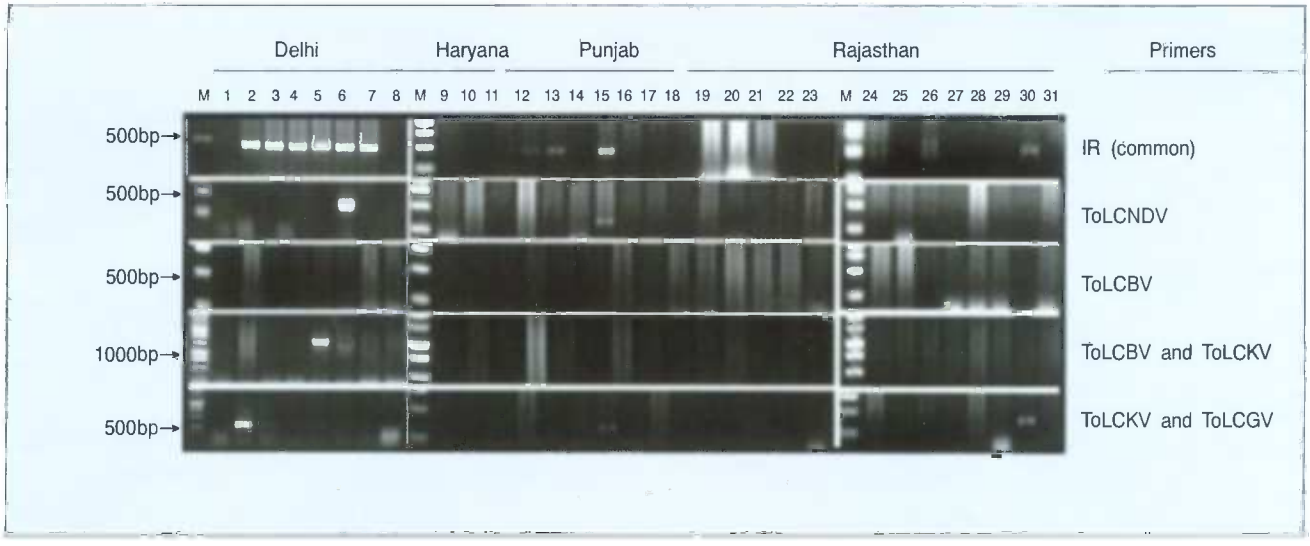

*Fig. 5: Agarose gel electrophoresis of PCR amplified products from samples collected from north-western region tested with common and species specific primers to tomato leaf curl begomoviruses. Lane M-1 Kb DNA ladder; Lane 1-*Coleus *sp.; Lane 2-* Datura metel; *Lane 3-*Eclipta alba; *Lane 4-*Tagetes erecta; *Lane 5-*Parthenium hysterophorus; *Lane 6-*Phyllanthus niruri; *Lane 7-*Solanum nigrum; *Lane 8-*Tabernaemontana divaricata; *Lane 9-*P. hysterophorus; *Lane 10-*Corchorus *sp.; Lane 11-*Croton bonplantianum; *12-*Tribulus terrestris; *Lane 13-*Cucumis *sp.; Lane 14-*Corchorus *sp.; Lane 15-*Solanum nigrum; *Lane 16-*Trianthima portulacastrum; *Lane 17-*Digeria arvensis; *Lane 18-*E. alba; *Lane 19-chilli-1; Lane 20-*chilli-2; *Lane 21-*Zatropa curcus; *Lane 22-*E.alba; *Lane 23-*castor; *Lane 24-*moth bean; *Lane 25-*Amaranthus *sp.; Lane 26-* cluster bean; *Lane 27-*T. portulacastrum; *Lane 28-*C. bonplantianum; *Lane 29-*P. hysterophorus; *Lane 30-*wild sunflower; *Lane 31-*Xanthium strumarium

amplified with the common IR primers. However, none of the samples from Haryana amplified with any of the IR primers. Using virus specific primers, ToLCNDV was detected in samples of *S. nigrum* from Delhi and Abohar (Fig. 5, lane 7 and 15). *D. metel* from Delhi and wild sunflower from Sri Ganganagar were positive for ToLCGV (Fig. 5, lane 2 and 30). ToLCBV and ToLCKV were not detected in any of the samples tested. Interestingly, samples of *E. alba*, *T. erecta*, *P. hysterophorus* and *P. niruri* originating from Delhi, and *Tribulus terrestris* and *Cucumis* sp. originating from Abohar amplified with the common IR primers but not with any of the four sets of species specific primers. Castor, chilli and mothbean originating from Sri Ganganagar and *Coleus* sp. originating from Delhi were not PCR positive for any of the four begomoviruses, which cause TomLCD in India.

DISCUSSION

WTGs known to cause TomLCD in India have a restricted

Table 2. PCR detection of begomoviruses, known to cause TomLCD in India, in weed and other plant species collected from areas adjoining tomato fields

Origin of samples	Symptoms*	Detection by the common IR primers	ToLCNDV	ToLCBV	ToLCKV	ToLCGV
Delhi						
Coleus sp.	LC	–	–	–	–	–
Datura metel	LC	+	–	–	–	+
Eclipta alba	YV	+	–	–	–	–
Tagetes erecta	UC, R	+	–	–	–	–
Parthenium hysterophorus	LC, SB	+	–	–	–	–
Phyllanthus niruri	LC, YM	+	–	–	–	–
Solanum nigrum	YM, LCr	+	+	–	–	–
Tabernaemontana divaricata	LCr, M	–	–	–	–	–
Haryana (Hisar)						
P. hysterophorus	SL, MC	–	–	–	–	–
Corchorus sp.	LC	–	–	–	–	–
Crotons sp.	YV	–	–	–	–	–
Punjab (Abohar)						
Tribulus terrestris	NS	+	–	–	–	–
Cucumis sp.	YM	+	–	–	–	–
Corchorus sp.	LC	–	–	–	–	–
Solanum nigrum	LCr, M	+	+	–	–	–
Trianthima portulacastrum	NS	–	–	–	–	–
Digeria arvensis	YM	–	–	–	–	–
Rajasthan (Sri Ganganagar)						
E.alba	YV	–	–	–	–	–
Chilli -1	LC	–	–	–	–	–
Chilli-2	LC	–	–	–	–	–
Zatropa curcus	C	–	–	–	–	–
E. alba	YV	–	–	–	–	–
Castor	DC	–	–	–	–	–
Moth bean	YM	–	–	–	–	–
Amaranthus sp	NS	–	–	–	–	–
Cluster bean	YM	+	–	–	–	–
Trianthima portulacastrum	NS	–	–	–	–	–
Crotan bonplantianum	YV	–	–	–	–	–
P. hysterophorus	SL	–	–	–	–	–
Wild sunflower	YV	+	–	–	–	+
Xanthium strumarium	LCr, Y	–	–	–	–	–

* LC-leaf curl; MC-Mild curl; YM-yellow mosaic; DC-downward curl; C-cupping; M-mosaic; YV-yellow vein; R-rolling of leaves; SB-stem bending; LCr-leaf crinkle; SL-small sized leaves; Y-yellowing; NS-no phenotypic symptoms.

host range. In this study, WTGs from six (*D. metel, E. alba, T. erecta, P. hysterophorus, P. niruri* and *S. nigrum*) of the eight plant species tested from Delhi were transmitted to tomato cv. Pusa Ruby by whitefly and the infected tomato plants developed leaf curl symptoms with minor variations which showed that these plants are natural hosts of tomato infecting begomoviruses. Natural infection of ToLCNDV has been found in cowpea and croton, of ToLCKV in *P. hysterophorus* and tobacco, and of ToLCBV in okra (Reddy *et al.*, 2005), but natural infection of tomato infecting WTGs in *D. metel, E. alba, T. erecta, P. niruri* and *S. nigrum* has been demonstrated for the first time in this study. However, of these only *S. nigrum* was found to contain DNA A and DNA B whereas the other five non-tomato hosts contained only DNA A and DNA β indicating that *S. nigrum* is naturally infected by ToLCNDV, and the other five hosts are infected by some other WTGs. Natural infection by tomato infecting WTGs in *D. metel* and *S. nigrum* was expected as these are found to be susceptible to these viruses under glass house conditions (Gupta *et al.*, 2001). But, the WTGs known to cause TomLCD in India were detected only in two of the six plant samples from Delhi by PCR with virus species specific primers; ToLCNDV was detected in *S. nigrum* and ToLCGV in *D. metel*. ToLCNDV was also detected by PCR in sample of *S. nigrum* collected from Punjab. ToLCGV was also detected in wild sunflower collected from Rajasthan. ToLCNDV has been detected for the first time in *S. nigrum*, and ToLCGV in *D. metel* and wild sunflower. The other two WTGs causing TomLCD in India, ToLCBV and ToLCKV, were not detected in any of the plant species tested in this study indicating that these two viruses do not occur commonly in north-western India. However, in a similar study Reddy *et al.* (2005) detected ToLCKV in *P. hysterophorus* but not the other three WTGs in Karnataka. In the present study *P. hysterophorus* was found to be a natural host of some uncharacterized tomato infecting WTG, which may also be spreading to tomato crops. *P. hysterophorus* is the most widely distributed and most difficult weed to manage in India. As it also harbours WTGs, it may be a greater nuisance than realized so far. The findings of this study indicate that *D. metel, S. nigrum* and wild sunflower may be playing important role in the perpetuation of ToLCNDV and ToLCGV in northern India. ToLCNDV was also detected in crop plants like potato (results not shown) in this study; interestingly, it was not detected in chilli plants. Earlier workers have also detected ToLCNDV in crop plants like potato (Usharani *et al.*, 2004) and sponge gourd (Sohrab *et al.*, 2003). These and other crop plants, which have not been tested so far, could be important bridge hosts for the spread of ToLCNDV in tomato in northern India, where overlapping crops are common. This study provides the first report of (a) molecular detection of tomato infecting begomoviruses in *D. metel, E. alba, T. erecta, P. niruri* and *S. nigrum*, and (b) first report of *Begomovirus* infection in *T. divaricata*, and (c) indication of the occurrence of uncharacterized tomato infecting WTGs in northern India.

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