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Pathogen Profile

# *Tomato leaf curl New Delhi virus*: a widespread bipartite begomovirus in the territory of monopartite begomoviruses

SYED SHAN-E-ALI ZAIDI[1], DARREN P. MARTIN[2], IMRAN AMIN[1], MUHAMMAD FAROOQ[1] AND SHAHID MANSOOR[1,*]

[1]*National Institute for Biotechnology and Genetic Engineering, Jhang Road, Faisalabad. PO Box 577, Pakistan*

[2]*Institute of Infectious Diseases and Molecular Medicine, Department of Integrative Biomedical Sciences, Division of Computational Biology, University of Cape Town, Anzio Rd, Observatory, Cape Town, 7925, South Africa*

**SUMMARY:**

*Tomato leaf curl New Delhi virus* (ToLCNDV) is an exceptional Old World bipartite begomovirus. On the Indian subcontinent, a region in which monopartite DNA satellite-associated begomoviruses with mostly narrow geographical ranges predominate, it is widespread, with a geographical range also including the Far East, Middle East, North Africa and Europe. The success of ToLCNDV probably derives from its broad host range and highly flexible genomic configuration: its DNA-A component is capable of productively interacting with, and trans-replicating, diverse DNA-B components and betasatellites. An understanding of the capacity of ToLCNDV to infect a variety of hosts and spread across a broad and ecologically variable geographical range could illuminate the potential economic threats associated with similar begomoviral invasions. Towards this end, we used available ToLCNDV sequences to reconstruct the history of ToLCNDV spread.

**Taxonomy:** Family *Geminiviridae*, Genus *Begomovirus*. ToLCNDV is a bipartite begomovirus. Following the revised begomovirus taxonomic criteria of 91% and 94% nucleotide identity for species and strain demarcation, respectively, ToLCNDV is a distinct species with two strains: ToLCNDV and ToLCNDV-Spain.

**Host range:** The primary cultivated host of ToLCNDV is tomato (*Solanum lycopersicum*), but the virus is also known to infect 43 other plant species from a range of families, including Cucurbitaceae, Euphorbiaceae, Solanaceae, Malvaceae and Fabaceae.

**Disease symptoms:** Typical symptoms of ToLCNDV infection in its various hosts include leaf curling, vein thickening, puckering, purpling/darkening of leaf margins, leaf area reduction, internode shortening and severe stunting.

**Keywords:** begomovirus replication, begomovirus resistance, geminivirus diversity, hypersensitive response, pseudorecombination, *Tomato leaf curl New Delhi virus*.

* *Correspondence*: Email: Shahidmansoor7@gmail.com

## INTRODUCTION

Tomato leaf curl disease (ToLCD) has emerged as one of the most devastating diseases of tomato on the Indian subcontinent and can cause total yield loss in plants infected at a young age (Chakraborty, 2008; Prasanna *et al.*, 2015). ToLCD was first reported from India in 1948 (Vasudeva and Raj, 1948) and is, today, known to occur elsewhere in Asia and the Pacific Rim, in north Africa and in Europe. ToLCD is also a major tomato disease in sub-Saharan Africa, where it is associated with a complex of several monopartite begomoviruses (Abhary *et al.*, 2007; Kon and Gilbertson, 2012; Leke *et al.*, 2011; Osei *et al.*, 2008; Zhou *et al.*, 2008). On the Indian subcontinent, at least 13 distinct virus species have been associated with ToLCD (Brown *et al.*, 2015), with one of the most important being *Tomato leaf curl New Delhi virus* (ToLCNDV). In addition to infecting elite tomato cultivars (Sahu *et al.*, 2010), ToLCNDV also infects at least 43 other dicotyledonous plant species, including weeds and economically important vegetable and ornamental species (Table S1, see Supporting Information). Another important and widespread leaf curl disease of tomato is tomato yellow leaf curl disease (TYLCD) (Abhary *et al.*, 2007), which is associated with a set of begomoviruses collectively referred to as tomato yellow leaf curl viruses (TYLCVs) (Brown *et al.*, 2015). The intercellular dynamics and evolution of the TYLCV complex have been extensively reviewed in Gafni (2003) and Diaz-Pendon *et al.* (2010), respectively. In this article, however, we focus on ToLCD specifically with reference to ToLCNDV.

ToLCNDV is a member of the *Geminiviridae* family of plant viruses. Geminiviruses are characterized by their quasi-icosahedral twinned particles that are approximately $18 \times 30$ nm$^2$ in size and encapsidate circular, single-stranded (ss) DNA of ~2.5–3.1 kb (Stanley, 1985). Based on their host ranges, insect vectors and genome organizations, geminiviruses are classified into seven genera, including *Begomovirus*, *Curtovirus*, *Topocuvirus*, *Mastrevirus*, *Becurtovirus*, *Turncurtovirus* and *Eragrovirus* (King *et al.*, 2012; Varsani *et al.*, 2014). However, most of the economically important geminiviruses are members of the genus *Begomovirus*. There are 288 species of begomoviruses (www.ictvonline.org)

which, based on their genome organization, are classified into two groups: monopartite if they have a single genome component, and bipartite if they have two genome components. Begomoviruses are transmitted by the sweet potato/tobacco/silver leaf whitefly, *Bemisia tabaci* (Gennadius) (Order: Hemiptera, Family: Aleyrodidae), in a circulative persistent manner, and are mostly restricted to the phloem of infected plants (reviewed in Gilbertson *et al.*, 2015 and Navas-Castillo *et al.*, 2011). They infect important dicotyledonous crops of several families, including Cucurbitaceae (gourds, squash, watermelon and melon), Euphorbiaceae (cassava), Solanaceae (tobacco, petunia, chilli, tomato and potato), Malvaceae (okra, cotton) and Fabaceae (cowpea, mung bean, common bean, lima bean and soybean) in different regions of the world (Seal *et al.*, 2006).

## GENOME ORGANIZATION AND PROTEINS

ToLCNDV is a bipartite begomovirus in that it has two genome components, named DNA-A and DNA-B (Padidam *et al.*, 1995). DNA-A contains the AV1 and AV2 genes in the virion sense orientation, and AC1, AC2, AC3 and AC4 in the complementary sense orientation (Padidam *et al.*, 1996). ToLCNDV DNA-B contains the BV1 gene in the virion sense orientation, and the BC1 gene in the complementary sense orientation (Fig. 1A; Padidam *et al.*, 1995). The genes in the virion and complementary sense orientations on DNA-A and DNA-B are separated by an intergenic region containing a common region (CR) comprising sequences that are conserved between DNA-A and DNA-B. The main topological feature of the CR is a hairpin structure with a conserved nonanucleotide (TAATATT/AC) that spans the virion strand origin of replication (*v-ori*, indicated by '/') (Padidam *et al.*, 1995). Iterated ~5–7-nucleotide-long sequences (called iterons) that are present 5′ of the hairpin form binding sites for the virus replication-associated protein, Rep (encoded by AC1). In addition to a Rep protein, the ToLCNDV DNA-A encodes a replication enhancer protein [REn; from AC2 open reading frame (ORF)], a transcriptional activator protein (TrAP; from AC3), a coat protein (CP; from AV1), an AV2 protein and an AC4 protein. DNA-B encodes a movement protein (MP; from BC1) and a nuclear shuttle protein (NSP; from BV1) (Fondong, 2013).

ToLCNDV is also often associated with betasatellites (Akhter *et al.*, 2014; Jyothsna *et al.*, 2013a,b; Shafiq *et al.*, 2010; Singh *et al.*, 2012, 2016). Betasatellites are half the size (approximately 1.4 kb) of virus DNA-A and DNA-B components. Their most conserved features are the presence of a single gene, betaC1, encoding a pathogenicity determinant protein (Hanley-Bowdoin *et al.*, 2013), an adenine-rich region and a conserved region that contains a hairpin very similar to that at the DNA-A and DNA-B *v-ori* sequences (complete with the same conserved nonanucleotide).

Betasatellites and DNA-B depend on the Rep encoded by DNA-A for their replication (Hanley-Bowdoin *et al.*, 2013;

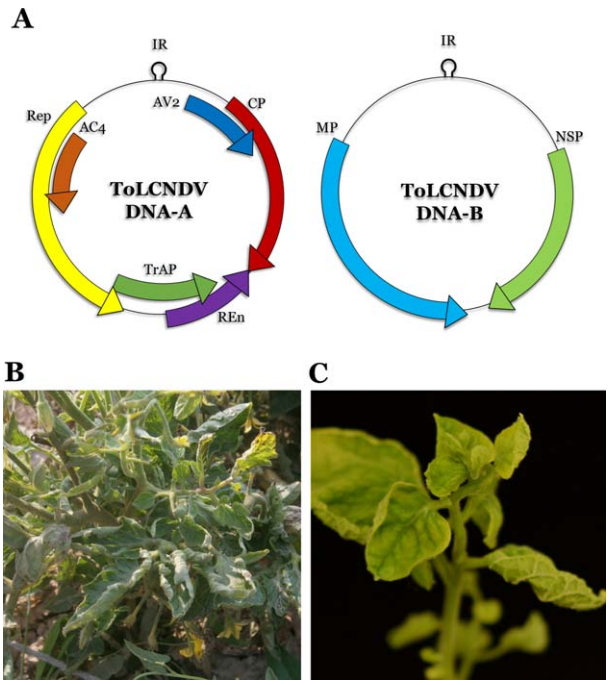



**Fig. 1** Genome organization of *Tomato leaf curl New Delhi virus* (ToLCNDV) and tomato leaf curl disease symptoms. (A) ToLCNDV genomic components DNA-A and DNA-B with arrows showing their respective genes. Genes on DNA-A encode a replication-associated protein (Rep), a replication enhancer protein (REn), a transcriptional activator protein (TrAP), a coat protein (CP), an AV2 protein and an AC4 protein; genes on DNA-B encode a movement protein (MP) and a nuclear shuttle protein (NSP). (B) Tomato leaf curl disease-affected tomato plant in the field with typical disease symptoms. (C) Symptoms of ToLCNDV infection on a *Nicotiana benthamiana* plant.

Heyraud-Nitschke *et al.*, 1995). Rep contains three main domains: a DNA-binding domain, an oligomerization domain and a helicase domain (Fondong, 2013). The interaction between the oligomerization domain and Rep-binding domain facilitates virus accumulation (Chatterji *et al.*, 2001). Two major factors determining the specificity with which the ToLCNDV Rep is able to replicate DNA-A, DNA-B and betasatellite molecules are an iteron-related domain (IRD) in the N-terminus of Rep and the sequences of iterons within the Rep-binding region 5′ of *v-ori* (Chatterji *et al.*, 1999, 2000). Following recognition of appropriate iteron sequences by the Rep IRD, Rep binds near *v-ori* (Behjatnia *et al.*, 1998) and initiates rolling circle replication (RCR) (Laufs *et al.*, 1995).

With a coding capacity of only a few genes, geminiviruses mostly rely on their host's DNA polymerases and accessory factors for replication inside the nucleus. The proteins encoded by geminiviruses influence several cellular pathways of their host, including RNA silencing, cell differentiation, cell cycle control and plasmodesmata function (Hanley-Bowdoin *et al.*, 2013). The ToLCNDV NSP triggers a hypersensitive response (HR) in infected plants, which suggests that it may be a pathogenicity determinant (Hussain *et al.*, 2005; Malik *et al.*, 2011), i.e. the HR in plants is a

mechanism of virus resistance that inhibits virus spread via the induction of programmed cell death. The N-terminus of ToLCNDV TrAP contains a zinc finger domain and a nuclear localization signal (NLS), and is also potentially involved in overcoming host defences via the inhibition of the HR (Hussain *et al.*, 2007). The CP and AV2 proteins of ToLCNDV are involved in virus movement and their absence results in reduced levels of ssDNA (Padidam *et al.*, 1996, 1999). The CP of ToLCNDV also interacts with a midgut protein of *B. tabaci* and probably facilitates virus transport both from the digestive tract to the haemolymph, and from the haemolymph to the salivary glands (Rana *et al.*, 2016).

## GEOGRAPHICAL DIVERSITY AND HOST RANGE

In the Old World (OW), monopartite begomoviruses with associated betasatellites are apparently more common than bipartite begomoviruses (Briddon *et al.*, 2014). ToLCNDV is of specific interest because of its bipartite nature and extensive host range in the OW. It was initially identified on solanaceous crops in India (Padidam *et al.*, 1995), but has since been reported infecting 43 diverse plant species in Pakistan, India, Bangladesh, Iran, Sri Lanka, Malaysia, Taiwan, Thailand, Indonesia, Tunisia, Spain and Italy (Fig. 2A,B; a comprehensive list of all the reported hosts of ToLCNDV in different countries is given in Table S1). In potato (*Solanum tuberosum*), ToLCNDV has been associated with potato apical leaf curl disease (Garg *et al.*, 2001; Usharani *et al.*, 2004) on commercial potato varieties in India (Chandel *et al.*, 2010). In September 2012, ToLCNDV was identified, for the first time, in leaf curl disease-affected cucurbits in Murcia, Spain, with similar symptoms being observed in Almeria in May 2013 and throughout southern Spain by autumn 2013, where it was found infecting both glasshouse and open-field crops (López *et al.*, 2015). In early 2015, severe damage of plastic tunnel-cultivated zucchini, cucumber and melon by ToLCNDV was reported in the Kébili region of south-eastern Tunisia (Mnari-Hattab *et al.*, 2015). By autumn 2015, similar symptoms were observed in horticultural areas of Sicily and southern Italy (Panno *et al.*, 2016). Collectively, these reports suggest that ToLCNDV has been introduced only recently to North Africa and southern Europe.

A consideration of ToLCNDV phylogeny in the context of the host species and locations in which virus isolates have been sampled, called a phylogeographical analysis, could potentially illuminate links between its geographical distribution, host range and evolution (Fig. 3). The phylogenetic analysis was performed on a multiple sequence alignment of full-length ToLCNDV sequences. It is noteworthy, however, that, among all European ToLCNDV accessions, only five were full length (Fig. 2A; Table S1). Nevertheless, partial ToLCNDV genome sequences from Tunisia and Italy share high degrees of nucleotide identity (97.6%–99.2%) with the Spanish isolates, such that it is very likely that the Italian and Tunisian isolates are monophyletic with the Spanish isolates.

The phylogenetic tree indicates that ToLCNDV isolates from the Indian subcontinent are evolutionarily distinct from ToLCNDV variants recently reported from Spain. The isolates from Spain are monophyletic and form a nested clade within a much more diverse group of sequences from the Indian subcontinent; this pattern strongly indicates that the Spanish ToLCNDV population was founded by a single virus that originated on the Indian subcontinent. This pattern is similar to that displayed by the ToLCNDV isolates from Thailand, Indonesia and Taiwan. These isolates, although more diverse than those sampled in Spain, also form a monophyletic group of isolates (Fig. S1, see Supporting Information), which is nested within a larger and more diverse group of isolates from the Indian subcontinent. Therefore, it can be concluded that the Spanish (and possibly also the entire European/North African) population of ToLCNDV was founded by a single virus isolate.

Unlike the clear geographical structuring of the ToLCNDV phylogeny, there is no obvious association between the host species from which viruses have been sampled and their phylogenetic placement (Venkataravanappa *et al.*, 2015).

## PSEUDO-RECOMBINATION AND COMPONENT CAPTURE

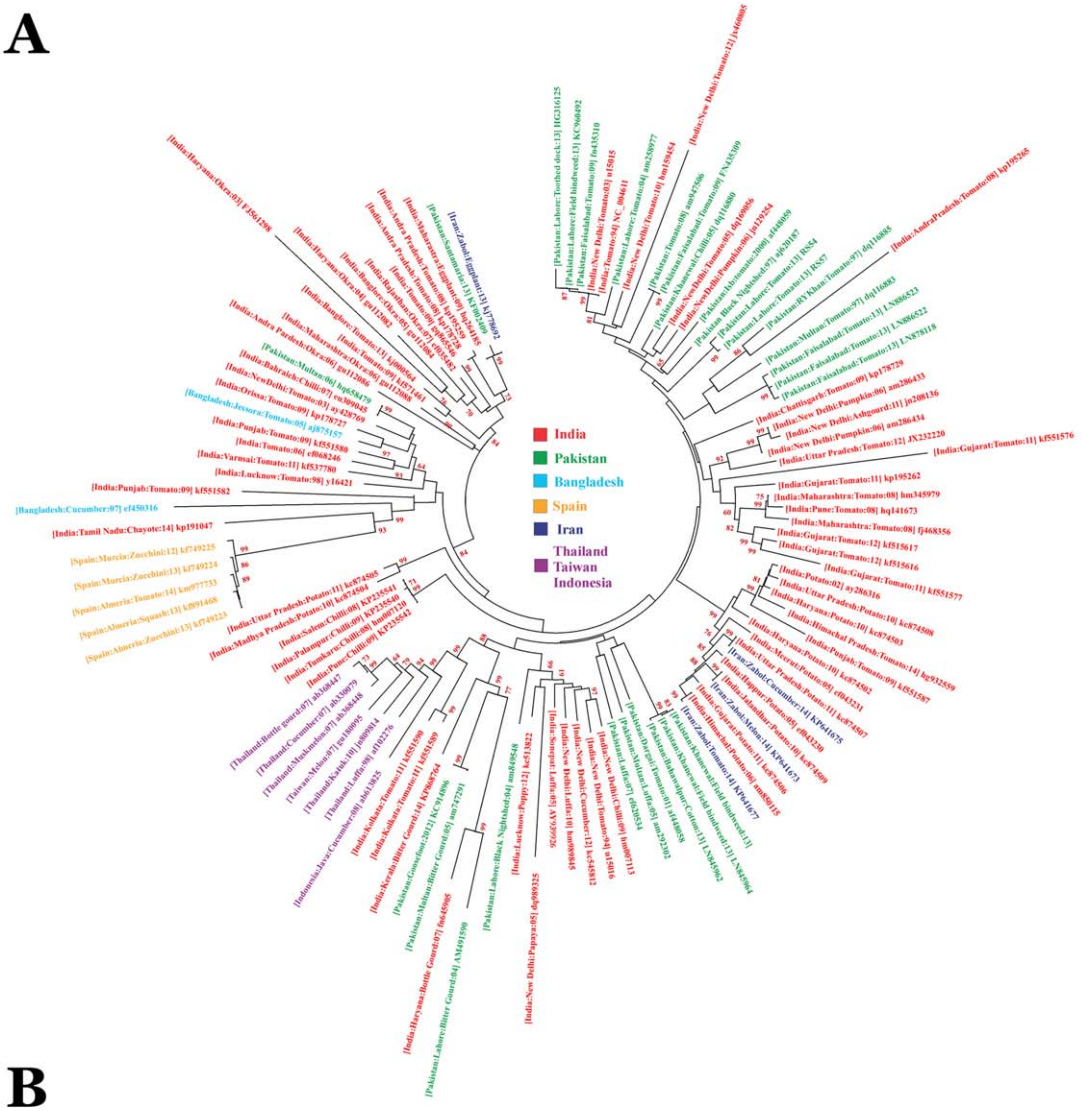
Re-assortment of genomic components/segments among multipartite viruses is known as pseudo-recombination, and the capture of one or more genomic components/satellites of a multipartite virus by another virus is known as component capture. Re-assortment and component capture are both apparently very common in begomoviruses and are probably both major mechanisms whereby begomoviruses adapt to infect new hosts (Lefeuvre and Moriones, 2015). ToLCNDV DNA-A and DNA-B components have been known to interact with several viruses and betasatellites within the context of mixed infections in a variety of host species (Table 1).

Pseudo-recombination of ToLCNDV with *Tomato leaf curl Palampur virus* (ToLCPalV) has been observed both in the field and experimentally (Malik *et al.*, 2011). The trans-encapsidation (the transmission of one virus genome within the capsid encoded by a different virus genome) and whitefly transmission of ToLCPalV DNA-B, mediated by the CP of ToLCNDV, has also been demonstrated (Kanakala *et al.*, 2013).

ToLCNDV infects chillies (*Capsicum annum*) in Pakistan (Hussain *et al.*, 2004) and India (Khan *et al.*, 2006) and, in so doing, has been found to interact with *Pepper leaf curl Lahore virus* (PepLCLV) (Shafiq *et al.*, 2010) and *Chilli leaf curl virus* (ChLCV) (Singh *et al.*, 2016).

The DNA-A of *Tomato leaf curl Gujarat virus* (ToLCGuV) can trans-replicate the DNA-B of ToLCNDV and vice versa

**A**




**B**

| India | | Pakistan | | Indonesia | |
|---|---|---|---|---|---|
| *Ageratum* spp. | *Datura stramonium* | *Momordica charantia* | *Chenopodium album* | *Capsicum annuum* | *Cucumis sativus* |
| *Benincasa hispida* | *Luffa cylindrica* | *Convolvulus arvensis* | *Luffa cylindrica* | **Thailand** | |
| *Momordica charantia* | *Aleyrodes brassicae* on *Catharanthus roseus* | *Solanum nigrum* | *Cestrum nocturnum* | *Sauropus androgynus* | *Luffa cylindrica* |
| *Lagenaria siceraria* | *Carica papaya* | *Capsicum annuum* | *Parthenium hysterophorus* | *Lagenaria siceraria* | *Cucumis melo* |
| *Daucus carota* | *Catharanthus roseus* | *Gossypium hirsutum* | *Solanum lycopersicum* | *Cucumis sativus* | |
| *Saccharum edule* | *Papayer somniferum* | *Eclipta prostrata* | *Rumex dentatus* | **Spain** | |
| *Capsicum annuum* | *Solanum tuberosum* | | | *Cucurbita* spp. | *Cucurbita pepo* |
| *Cucumis sativus* | *Cucurbita pepo* | **Bangladesh** | | *Solanum lycopersicum* | |
| *Solanum melongena* | *Luffa cylindrica* | *Cucumis sativus* | *Solanum lycopersicum* | **Tunisia** | |
| *Crossandra infundibuliformis* | *Trichosanthes cucumerina* | **Iran** | | *Cucumis melo* | *Cucurbitaceae* family |
| *Cyamopsis tetragonoloba* | *Luffa aegyptiaca* | *Cucumis sativus* | *Capsicum annuum* | **Sri Lanka** | |
| *Coccinia grandis* | *Nicotiana tabacum* | *Solanum melongena* | *Solanum lycopersicum* | *Momordica charantia* | *Luffa* spp. |
| *Jasminum multiflorum* | *Solanum lycopersicum* | *Cucumis melo* | | *Trichosanthes cucumerina* | *Cucurbita pepo* |
| *Jatropha* spp. | *Citrullus lanatus* | **Taiwan** | | **Italy** | |
| *Hibiscus cannabinus* | *Abelmoschus esculentus* | *Cucumis melo* | | *Luffa* spp. | *Cucumis sativus* |

**Fig. 2** A phylogenetic tree depicting the evolutionary relationships between *Tomato leaf curl New Delhi virus* (ToLCNDV) isolates sampled in different countries. (A) The phylogenetic tree is based on multiple sequence alignment of full-length ToLCNDV DNA-A sequences, with bootstrap support for individual branches determined with 1000 bootstrap replicates. Alignment was performed using the CLUSTALW method and the phylogenetic tree was constructed using the neighbour-joining method as implemented in MEGA 6.0 (Tamura *et al.*, 2013). The isolate descriptors are country, area, host, year and accession number. The sequences reported from Spain form a distinct monophyletic cluster (in light orange), as do the sequences from Indonesia, Thailand and Taiwan (in purple). (B) A comprehensive list of all hosts and countries from which ToLCNDV has been reported.

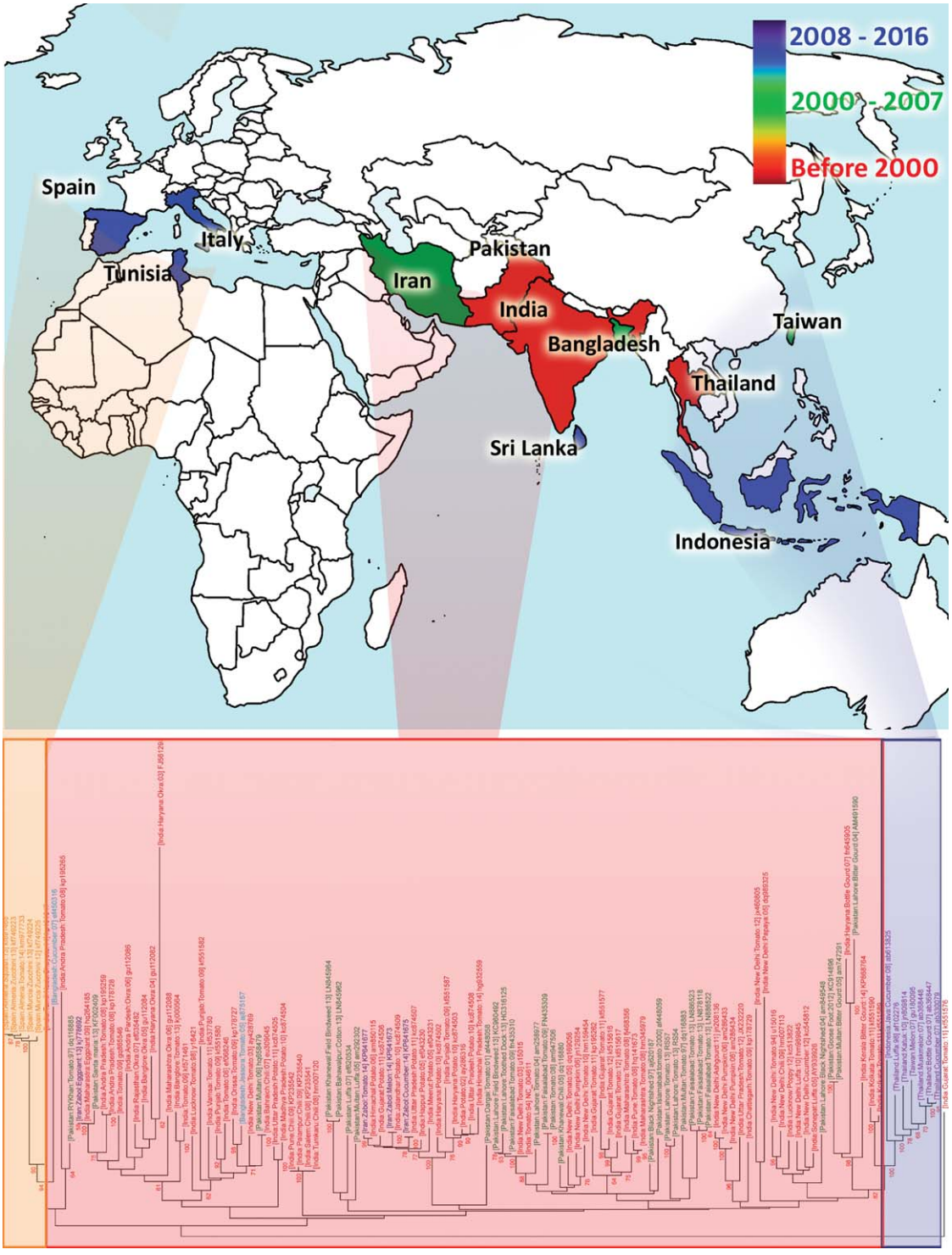



**Fig. 3** The distribution of *Tomato leaf curl New Delhi virus* (ToLCNDV) over time. Countries are coloured, according to the colour index (top right), with respect to the first report of ToLCNDV in the countries. The phylogenetic tree at the bottom is based on ToLCDNV DNA-A, and a spotlight on each cluster indicates the geographical region from which the isolates in that cluster originate.

**Table 1** *Tomato leaf curl New Delhi virus* interaction with various viruses and betasatellites in the context of mixed infection, component capture and synergistic interaction.

| ToLCNDV component | Interacting virus | Interacting betasatellite | Field host | Experimental host | Reference |
|---|---|---|---|---|---|
| DNA-A and DNA-B | CLCuKoV-Bur | CLCuMuB | *Gossypium hirsutum* | | Zaidi *et al.* (2016a) |
| DNA-A | ChLCV-A and ToLCGuV-B | TbLCB | *Capsicum annum* and *Capsicum chinense* | *Nicotiana benthamiana* and *Capsicum annum* | Singh *et al.* (2016) |
| DNA-A | ZYMV | | *Momordica charantia* | | Sharma S *et al.* (2015) |
| DNA-B | BYVMV | | *Abelmoschus esculentus* | | Venkataravanappa *et al.* (2015) |
| DNA-B | ToLCGuV-A | | *Gossypium hirsutum* | | Zaidi *et al.* (2015) |
| DNA-A and DNA-B | | ChLCB | | *Nicotiana benthamiana* | Akhter *et al.* (2014) |
| DNA-A and DNA-B | | CLCuMuB | *Solanum lycopersicum* | *Nicotiana benthamiana* and *Solanum lycopersicum* | Jyothsna *et al.* (2013a) |
| DNA-A and DNA-B | | TYLCTHB | *Solanum lycopersicum* | | Jyothsna *et al.* (2013a) |
| DNA-A and DNA-B | | PaLCuB | *Solanum lycopersicum and Solanum tuberosum* | | Jyothsna *et al.* (2013a) |
| DNA-A and DNA-B | | BYVB | *Solanum tuberosum* | | Jyothsna *et al.* (2013a) |
| DNA-A and DNA-B | | LuLDB | *Luffa cylindrica, Momordica charantia, Cucumis sativus and Lagenaria siceraria* | *Nicotiana benthamiana* | Jyothsna *et al.* (2013a) |
| DNA-B | ToLCGuV-A | | | *Nicotiana benthamiana* and *Solanum lycopersicum* | Jyothsna *et al.* (2013b) |
| DNA-B | ToLCGuV-A | TYLCTHB | | *Nicotiana benthamiana* and *Solanum lycopersicum* | Jyothsna *et al.* (2013b) |
| DNA-A and DNA-B | ToLCPalV-A and ToLCPalV-B | | *Solanum lycopersicum* | *Solanum lycopersicum* | Kanakala *et al.* (2013) |
| DNA-B | ToLCPalV-A | | | *Solanum lycopersicum* and *Cucumis sativus* | Kanakala *et al.* (2013) |
| DNA-A | ToLCPalV-B | | | *Solanum lycopersicum* and *Cucumis sativus* | Kanakala *et al.* (2013) |
| DNA-A | SLCCNV and ToLCPalV | | *Cucurbita moschata* | | Jaiswal *et al.* (2012) |
| DNA-A | | RaLCB | | *Nicotiana benthamiana* and *Solanum lycopersicum* | Singh *et al.* (2012) |
| DNA-B | RLCuV | | | *Nicotiana benthamiana* and *Solanum lycopersicum* | Singh *et al.* (2012) |
| DNA-B | CYVMV | | | *Nicotiana benthamiana* and *Solanum lycopersicum* | Singh *et al.* (2012) |
| DNA-A | CYVMV | CYVMB | | *Nicotiana benthamiana* and *Solanum lycopersicum* | Singh *et al.* (2012) |
| DNA-A and DNA-B | CYVMV | | | *Nicotiana benthamiana* and *Solanum lycopersicum* | Singh *et al.* (2012) |
| DNA-A and DNA-B | CYVMV | CYVMB | | *Nicotiana benthamiana* and *Solanum lycopersicum* | Singh *et al.* (2012) |
| DNA-B | ToLCRnV | | | *Nicotiana benthamiana* and *Solanum lycopersicum* | Kumari *et al.* (2011) |
| DNA-A | | ToLCRnB | | *Nicotiana benthamiana* | Kumari *et al.* (2011) |
| DNA-B | ToLCPalV-A | | *Cucurbita melo* | *Nicotiana benthamiana* | Malik *et al.* (2011) |

**Table 1** *Continued*

| ToLCNDV component | Interacting virus | Interacting betasatellite | Field host | Experimental host | Reference |
|---|---|---|---|---|---|
| DNA-B | PepLCLV | ChLCB | *Capsicum annum* | *Nicotiana benthamiana*, *Nicotiana tabacum* and *Capsicum annum* | Shafiq *et al.* (2010) |
| DNA-B | PepLCLV | | | *Nicotiana benthamiana*, *Nicotiana tabacum* and *Capsicum annum* | Shafiq *et al.* (2010) |
| DNA-A | ToLCGuV-B | | | *Nicotiana benthamiana*, *Nicotiana tabacum* and *Solanum lycopersicum* | Chakraborty *et al.* (2008) |
| DNA-B | ToLCGuV-A | | | *Nicotiana benthamiana*, *Nicotiana tabacum* and *Solanum lycopersicum* | Chakraborty *et al.* (2008) |

*Bhendi yellow vein mosaic virus* (BYVMV), bhendi yellow vein betasatellite (BYVB), *Chilli leaf curl virus* (ChLCV), chilli leaf curl betasatellite (ChLCB), *Cotton leaf curl Kokhran virus*-Burewala strain (CLCuKoV-Bur), cotton leaf curl Multan betasatellite (CLCuMuB), *Croton yellow vein mosaic virus* (CYVMV), croton yellow vein mosaic betasatellite (CYVMB), luffa leaf distortion betasatellite (LuLDB), papaya leaf curl betasatellite (PaLCuB), *Pepper leaf curl Lahore virus* (PepLCLV), *Radish leaf curl virus* (RLCuV), radish leaf curl betasatellite (RaLCB), *Squash leaf curl China virus* (SLCCNV), tobacco leaf curl betasatellite (TbLCB), *Tomato leaf curl Gujarat virus* (ToLCGuV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Palampur virus* (ToLCPalV), tomato leaf curl Ranchi betasatellite (ToLCRnB), *Tomato leaf curl Ranchi virus* (ToLCRnV), tomato yellow leaf curl Thailand betasatellite (TYLCTHB), *Zucchini yellow mosaic virus* (ZYMV).

(Chakraborty *et al.*, 2008; Jyothsna *et al.*, 2013b). Mixed infections of ToLCNDV with ToLCGuV have also been observed in the field (Singh *et al.*, 2016; Zaidi *et al.*, 2015) and demonstrated experimentally (Chakraborty *et al.*, 2008; Jyothsna *et al.*, 2013b; Singh *et al.*, 2016).

Mixed infection of ToLCNDV with monopartite begomoviruses, such as *Bhendi yellow vein mosaic virus* (BYVMV) (Venkataravanappa *et al.*, 2015), *Cotton leaf curl Kokhran virus*-Burewala strain (CLCuKoV-Bur) (Zaidi *et al.*, 2016a), *Croton yellow vein mosaic virus* (CYVMV) (Singh *et al.*, 2012), *Squash leaf curl China virus* (SLCCNV) and *Tomato leaf curl Ranchi virus* (ToLCRnV) (Kumari *et al.*, 2011), are also common. Most of these interactions are synergistic in the sense that mixed infections with these viruses yield enhanced symptoms. Recently, Sharma S *et al.* (2015) have also described the mixed infection of ToLCNDV with an aphid-transmitted potyvirus, *Zucchini yellow mosaic virus* (ZYMV), within bitter gourd in India. Collectively, these studies indicate that mixed infections between ToLCNDV and other begomoviruses are common and probably enhance virus evolution via recombination and component capture.

Some studies have suggested that monopartite begomoviruses, such as BYVMV and CLCuKoV-Bur, have captured ToLCNDV DNA-B (Venkataravanappa *et al.*, 2015, Zaidi *et al.*, 2016b). The distinct evolutionary histories of the DNA-A and DNA-B components of bipartite begomoviruses probably indicate that, initially, DNA-B was possibly a satellite molecule, akin to a betasatellite, that was captured by a monopartite begomovirus, after which it became an integral component of begomovirus genomes (Briddon *et al.*, 2010).

Unlike for OW monopartite begomoviruses, only a few OW bipartite begomoviruses have been found to be associated with betasatellites. The first association of a bipartite begomovirus with a betasatellite was observed in tomato in 2004 (W. S. Tsai and S. K. Green, unpublished results; as cited by Bull *et al.*, 2004). Further emphasizing the unusual promiscuity of ToLCNDV is the fact that it has also been found to be associated with several betasatellites, such as chilli leaf curl betasatellite (ChLCB) (Akhter *et al.*, 2014), radish leaf curl betasatellite (RaLCB) (Singh *et al.*, 2012), tobacco leaf curl betasatellite (TbLCB) (Singh *et al.*, 2016), tomato leaf curl Ranchi betasatellite (ToLCRnB) (Kumari *et al.*, 2011), cotton leaf curl Multan betasatellite (CLCuMuB) (Sivalingam *et al.*, 2010) and CLCuMuB in combination with luffa leaf distortion betasatellite (LuLDB) (Jyothsna *et al.*, 2013a).

The association of ToLCNDV with these betasatellites apparently increases the severity of disease symptoms beyond that which is characteristically seen when only ToLCNDV DNA-A and DNA-B are present (Singh *et al.*, 2012). Although plants infected with just ToLCNDV DNA-A and a betasatellite, in the absence of DNA-B, can still display severe symptoms, symptoms become even more severe when all three molecules are present (Sivalingam and Varma, 2012). The probable role of the betasatellite in the absence of ToLCNDV DNA-B is the substitution of the DNA-B movement functions (Saeed *et al.*, 2007).

Given both the frequent involvement of ToLCNDV in mixed infection with various other begomovirus species, and the wide range of different betasatellites found to be associated with ToLCNDV, it is very likely that ToLCNDV is somewhat predisposed to the capture of the betasatellites of other begomoviruses. Although the selective processes underlying this predisposition are presently unknown, it is both likely that this predisposition is

evolutionarily beneficial, and plausible that it is beneficial because it has enabled ToLCNDV to expand its host range.

## VIRUS CONTROL STRATEGIES

Conventionally, begomovirus control strategies have focused on whitefly management employing pesticides, the activation of natural whitefly predators or the use of physical barriers, such as reflective mulches and UV-absorbing sheets (Legg *et al.*, 2014). In addition, several cultural practices, such as early sowing, weed management, crop-free periods, virus-free transplants and the removal of infected plants, have also been adopted for disease control. However, the complex epidemiological factors associated with begomovirus disease outbreaks, such as whitefly migration dynamics, rapid virus evolution and unpredictable virus host range expansions, have all made it very difficult to develop effective long-term disease management strategies (Loebenstein and Katis, 2014).

The most effective way of achieving this goal will probably be the development of begomovirus and/or whitefly immune plant genotypes and the use of these in combination with other control measures. Efforts are therefore continually ongoing both to understand the natural anti-begomovirus and anti-whitefly resistance mechanisms of plants that might be harnessed through either conventional breeding or genetic engineering approaches, and to devise completely artificial antiviral or insect defences that could be harnessed through genetic engineering.

Because of a lack of natural begomovirus resistance sources in cultivated tomato varieties, closely related wild species have been screened for resistance. Resistance to begomoviruses in tomato has been introgressed from wild *Solanum* species, such as *S. habrochaites*, *S. chilense*, *S. peruvianum* and *S. pimpinellifolium* (Prasanna *et al.*, 2015). Tomato cultivars in Bangladesh have also been screened for resistance to ToLCNDV (Maruthi *et al.*, 2005) and molecular markers tightly linked to ToLCNDV resistance in several tomato accessions have also been identified (Rai *et al.*, 2014). A number of different resistance genes have been further characterized from these various resistance sources, including *Ty-1* derived from *S. chilense* (Zamir *et al.*, 1994), *Ty-2* derived from *S. habrochaites* (Hanson *et al.*, 2000), *Ty-3* (Ji *et al.*, 2007) and *Ty-4* (Ji *et al.*, 2009) derived from *S. chilense*, and *ty-5* derived from the tomato cultivar 'Tyking' (Hutton *et al.*, 2012). Amongst the best characterized of these *Ty* genes are *Ty-1*, *Ty-3* and *ty-5*. Although *Ty-1* and *Ty-3* are allelic, encoding an RNA-dependent RNA polymerase (RDRP) (Verlaan *et al.*, 2013) which is involved in the RNAi response to viral infections, the *ty-5* locus is associated with a messenger RNA surveillance factor called Pelota (Lapidot *et al.*, 2015).

Most of the known resistance genes do not completely inhibit viral replication, but, instead, hinder viral accumulation to varying degrees (Verlaan *et al.*, 2013). As these different resistance genes confer different types of resistance, a rational approach would be to use multiple genes to produce durable and broad-spectrum resistance, a process known as pyramiding. Pyramiding *Ty-2* and *Ty-3* genes in tomato has been shown to increase resistance to ToLCNDV and other begomoviruses (both monopartite and bipartite) relative to when either gene is present alone (Prasanna *et al.*, 2015).

Efforts are also underway to produce ToLCNDV resistance in crops other than tomatoes. For example, ToLCNDV also causes severe disease in cucurbits, and resistant cucurbit cultivars of Indian origin have been identified by López *et al.* (2015). The virus is also a major cause of yellow mosaic disease in sponge gourd (*Luffa cylindrica*), and can cause 100% yield losses in infected plants (Islam *et al.*, 2011). Screening efforts in this species have revealed two cultivars, each with a single dominant gene controlling ToLCNDV resistance (Islam *et al.*, 2010). Sequence-related amplified polymorphisms (SRAPs) have also been used to screen for sponge gourd population markers that are linked to resistance against ToLCNDV, and two SRAP markers have been reported to be highly efficient in this regard (Islam *et al.*, 2011).

Recently, a comprehensive analysis of selected resistance-related genes (*R* genes) was carried out to investigate the basis of the molecular defence responses to ToLCNDV in several different hosts, including *Capsicum annuum*, *Solanum lycopersicum*, *Nicotiana tabacum* and *Nicotiana benthamiana*. Genes involved in both post-transcriptional gene silencing (such as RDR6, AGO1 and SGS3) and other defence-related processes (such as NSP interacting kinase or NIK, the nucleotide-binding site-leucine-rich repeat protein NBS-LRR and the lipid transfer protein LTP) display expression patterns that are substantially impacted by the presence of ToLCNDV (Kushwaha *et al.*, 2015). Of particular interest is NIK, a leucine-rich repeat receptor-like kinase (LRR-RLK), which triggers translational suppression on begomovirus infection, and has been found to show strong antiviral activity (Zorzatto *et al.*, 2015). Moreover, transgenic tomato plants with constitutively expressed NIK have been demonstrated to show high degrees of tolerance to tomato-infecting begomoviruses (Brustolini *et al.*, 2015).

RNA interference (RNAi) plays a critical role in the defence of plants against viruses. RNAi operates via two major classes of short RNAs: microRNAs (miRNAs) and small interfering RNAs (siRNAs; Karthikeyan *et al.*, 2013). miRNAs are endogenous non-coding RNAs which are produced by plants to regulate their own mRNA levels. During virus infections, miRNAs can trigger host defence mechanisms by the regulation of resistance genes. siRNAs, however, are derived from viral RNA. Overlapping viral RNA transcripts induce RNAi, leading to the degradation of viral mRNA and the production of siRNAs, a process often referred to as post-transcriptional gene silencing (PTGS). These virus-specific siRNA molecules target further degradation of viral RNA and also block translation. In addition, siRNAs homologous to the intergenic

regions of begomovirus genomes may result in the repression of transcription, a process often referred to as transcriptional gene silencing (TGS) (Vanitharani *et al.*, 2005). TGS and PTGS are both induced during virus infections, but these processes can also be induced artificially by targeting coding or non-coding sequences of viruses through the construction of a hairpin structure by designing specific target sequences in both the sense and anti-sense orientation, separated by an intron (Vanitharani *et al.*, 2005). When transcribed, such transcripts will produce siRNA molecules that are homologous to the target sequences (Kamthan *et al.*, 2015).

The role of short RNAs in defending plants against ToLCNDV infections has been studied by Sahu *et al.* (2010), who suggested that a decrease in ToLCNDV replicative intermediates within a ToLCNDV-tolerant cultivar may be correlated with the accumulation of virus-specific siRNAs. RNAi constructs targeting ToLCNDV AC1, AC2, AC4, AV1 and AV2 (Mubin *et al.*, 2007; Sharma V *et al.*, 2015; Vu *et al.*, 2013) show efficiently reduced virus accumulation by up to 90% (Sharma V *et al.*, 2015). However, profiling of the miRNA complements of healthy and ToLCNDV-infected tomato plants has revealed several novel miRNAs that probably function in ToLCNDV resistance (Naqvi *et al.*, 2010; Pradhan *et al.*, 2015), some of which could potentially be utilized to design virus resistance strategies.

Advanced technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR) associated (CRISPR/Cas) systems, can also be used to engineer broad-spectrum geminivirus resistance in plants (Ali *et al.*, 2015, 2016; Zaidi *et al.*, 2016b).

## CONCLUSIONS AND FUTURE PROSPECTS

1. ToLCNDV is a widespread bipartite begomovirus infecting more than 40 different plant species in 11 countries (Fig. 2). However, the spread of ToLCNDV to Europe and North Africa has probably occurred via the transfer of single founder viruses to southern Europe from the Indian subcontinent in the recent past (Fig. 3). Deeper sampling will be required to determine the precise origin of these founder viruses.
2. ToLCNDV co-occurs with several monopartite and bipartite begomoviruses and betasatellites, and may even participate in co-infections with viruses in other families (such as potyviruses). It is plausible, although currently unproven, that it is this interactive flexibility that is responsible for the wide host and geographical ranges of ToLCNDV (Table 1). Extensive work is presently underway to determine the molecular basis of inter-virus interactions (reviewed in Hanley-Bowdoin *et al.*, 2013), but the precise mechanistic details of the synergism of ToLCNDV with the wide array of viruses and satellites with which it is able to productively interact remain to be determined.
3. ToLCNDV is a serious threat to many economically important crops, such as tomato, potato, cucurbits and cotton. Based on its wide and apparently expanding geographical distribution, high levels of resistance against ToLCNDV would be extremely valuable. Strategies to achieve this objective might include conventional breeding strategies, such as *Ty* gene pyramiding, and molecular virus control strategies, such as engineered siRNA or guide RNAs (accompanied by scaffold RNA and Cas9) for CRISPR/Cas9, targeting virus conserved regions.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Fig. S1** The phylogenetic trees of *Tomato leaf curl New Delhi virus* (ToLCNDV) DNA-A (a) and DNA-B (b). Phylogenetic trees are based on full-length sequences, aligned in MUSCLE, MEGA 6. Different sequences reported from different countries are represented according to the colour index (top left of each tree). For both trees, branch supports have been tested with 1000 bootstrap replicates. The isolate descriptors are country, area, host, year and accession number.

**Table S1** Complete list of *Tomato leaf curl New Delhi virus* (ToLCNDV) sequences. Whenever multiple sequences have been reported from the same host, in the same year and from the same location, only one sequence has been listed.