Molecular identification of a *Begomovirus* associated with yellow vein net disease on *Malva parviflora* L. from india.

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Abstract

Incidence of yellow vein net disease with leaf distortion was observed on *Malva parviflora* grown as a weed in Barkatullah University campus, Bhopal, India during the rainy session. The *begomovirus* disease was suspected on the basis of symptomatology and whiteflies insects' population on the plant. The *begomovirus* was detected by the PCR with the *begomovirus* gene specific primers. The *begomovirus* under study showed highest nucleotide sequence identities and distinct phylogenetic relationships of coat protein gene (*CP*) with several isolates of Tomato leaf curl Kerala virus (ToLCKeV). This is the first report of ToLCKeV associated with yellow vein net disease on *M. parviflora* and it is a new host of *begomovirus* from India.

Keywords: *Malva parviflora*, *begomovirus*, Yellow vein net disease, Sequence identities, and Tomato leaf curl Kerala virus.

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Introduction

Malva parviflora L. (Family Malvaceae) commonly known as cheese weed and it is an exotic annual weed or perennial herb that is native to Northern Africa, Europe and Asia. It is used in the treatment of cough, throat infection and other bronchial problems as well as stomach and intestine irritations. The flowers and leaves are emollient and used for the softening of sensitive area of the skin. Combine with *Eucalyptus*; it makes a good remedy for cough and other chest ailments [1].

Madhya Pradesh is a central region of India and agriculture is one of the main sectors of the state's economy. About 73 percent population of the state is rural, which directly or indirectly depends on the agriculture. The Madhya Pradesh has plant diversity and flexible temperatures, which are favourable to the virus insect vector (aphids, whiteflies, leafhoppers, and planthoppers). There are few reports has been reported of the *begomoviruses* infection on *Jatropha gossypifolia* [2], *Solanum lycopersicum* [3] and *Cnidoscolus acontifolia* [4] from Madhya Pradesh state, India.

Begomoviruses is the largest genera in the family *Geminiviridae*. *Begomoviruses* have a circular, single-stranded deoxyribonucleic acid (ssDNA) genome and are transmitted in nature by the whitefly (*Bemisia tabaci*), and causes significant yield losses in economically important crop plants worldwide [5,6]. *Begomoviruses* generally have bipartite genomes (designated as DNA-A and DNA-B) and infect dicotyledonous plants. Based on their genome characteristics and phylogenetic relationships, *Begomoviruses* have been divided broadly into Old World (OW) viruses (Eastern hemisphere, Europe, Africa, Asia and Australasia) and the New World (NW) viruses (Western hemisphere, the Americas) [7,8]. Monopartite *begomoviruses* (have DNA-A genome only) are predominantly found in the Old World and are often associated with satellite DNAs (alpha-and betasatellites), which may or may not contribute to pathogenicity [9].

The first reported of the Malva veinal necrosis virus considered as belonging to the potato X virus group in *Malva parviflora* from Brazil [10]. In Israel, natural infection of Tomato yellow leaf curl virus-Isreal (TYLCV-Is) was found in the annual weed *M. Paraviflora* [11]. *M. parvifolia* acts as host for many viruses including the South African Cassava mosaic virus [12] and Faba bean necrotic yellow virus in Jordan [13]. In 2003, Antignus et al. reported that Squash leaf curl virus (SLCV) could infect *Malva nicaeensis* and *Ecballium elaterium* (Cucurbitaceae) in Israel [14]. Squash leaf curl virus (SLCV) was also found to occur naturally in *M. parviflora*, with severe leaf curling, yellowing and stunting of the whole plants. The full-length genomes of Squash leaf curl virus-Malva (SLCV-Malva) isolate were amplified using the bacteriophage F DNA polymerase enzyme [15]. Recently, the identified full-length *begomovirus* genome shared maximum nucleotide (nt) sequence identity at 92.5% with Hollyhock leaf curl virus (HoLCV), representing a new strain, *Ageratum* conyzoides symptomless alphasatellite (ACSLA) and *Ageratum* yellow vein India alphasatellite (AYVIA), new isolates of ACSLA and AYVIA identified from *M. parviflora* in Pakistan [16].

We report here, molecular detection and identification of a Tomato leaf curl Kerala virus associated with yellow vein net disease on *M. parviflora* from central region of Madhya Pradesh of India, based on sequence analysis of complete coat protein gene.

Materials and Methods

Virus source

Naturally infected *M. parviflora* plants showing severe yellow vein net disease with leaf distortion were collected from Barkatullah University campus, Bhopal, during rainy session (July) in year of 2016.

DNA extraction and polymerase chain reaction (PCR)

The total DNA was extracted from symptomatic and asymptomatic leaf samples of *M. parviflora* plants by Dellaporta et al. [17] method. To detection of *begomovirus*, the polymerase chain reactions (PCR) were carried out using total DNA as template and a set of *begomovirus* coat protein gene specific primers CPIT-I/CPIT-T (Acc. AM180920 and AM180921). The PCRs were set up in a 50 µL reaction mixture containing: template DNA (100 mg), dNTPs (10 mM each), primers (each 25 pmol), Taq DNA polymerase (1.5 U, Merk Pvt. Ltd), assay buffer 10X (Merk Pvt. Ltd) in a thermal cycler (Bio-Rad, USA). The PCR was done with the conditions: initial denaturation at 94°C for 5 min, followed by followed by 30 cycles of denaturation at 94°C for 1 min; annealing temperatures for 1 min at 47°C, extension at 72°C for 1.5 min and final extension cycle was 5 min at 72°C.

Sequence analysis of viral gene

The sequence data from the virus isolate under study were analysed by BLASTn (http:// www.ncbi.nlm.nih.gov/BLAST) and compared with existing sequences of *begomovirus* species available in the GenBank database. ORFs were translated into amino acids using the Expasy tool (http://www.expasy. org/ tools/dna.html). The sequence similarities of selected *begomovirus* species were obtained using the Genomatix DiAlign 2 program based on pair-wise alignment. Phylogenetic analyses were performed using the Molecular Evolutionary Genetics Analysis tool (MEGA v.7.1,) with 1000 replicates bootstrapping and the tree was generated with the Neighbour joining method.

Results

Disease symptoms

During a survey in rainy session July 2016, the *begomovirus*-like symptoms were observed on a large number of *M. parviflora* plants growing in Barkatullah University campus, Bhopal Madhya Pradesh with the disease incidence about 40%. The naturally infected plants exhibited severe yellow mosaic and leaf distortion symptoms (Figure 1). A population of whiteflies (*Bemisia tabaci*) was also noticed in the growing area therefore, association of *begomovirus* with the disease was suspected.

Figure 1. *Naturally infected malva parviflora weed plants. (a) A close view of a diseased plant howing symptoms of severe yellow vein net (b) as compared to healthy plant.*

Amplification of the begomovirus by PCR

The *begomovirus* was detected by PCR reactions using the total DNA as template and *begomovirus* coat protein gene specific primers: CPIT-I/CPIT-T. During PCR, the expected bands of ~800 bp were amplified from (3/3) naturally infected symptomatic plant samples but not in a healthy sample 1 (Figure 2). The PCR products (three samples) were purified by using PCR Clean-up System Kit (Promega, USA) and purified PCR product was sequenced. The consensus sequence data of three identical sequences were analyzed by the BLASTn and complete coat protein gene of 771 nucleotides of *begomovirus* under study isolate was deposited to GenBank database (Accession KY511140).

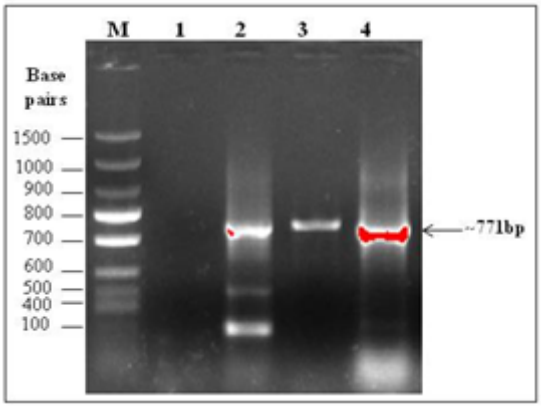

Figure 2. *1% Agarose gel electrophoresis image for detection of begomovirus by PCR using begomovirus coat protein gene specific primers, CPIT-I and CPIT-T, showing ~771 bp band in all naturally infected M. parviflora (lane 2-4) and but not in a negative control (DNA isolated from a healthy sample, lane 1). M=100 bp DNA Ladder DNA as Marker.*

Sequence analysis and Phylogeny of under study virus isolate

BLASTn analysis of the coat protein gene of *M. parviflora* (KY511140) of the *begomovirus* isolate from *M. parviflora* revealed highest 95-98% sequence identity with several isolates of Tomato leaf curl Kerala virus (ToLCKeV) on *Solanum lycopersicum* (KF551575, EU910140, EU910141, LT556075, LN886521, KY216063) and *Brassica rapa*

(KX671963) from India and Pakistan. The isolate also shared 94% identities with isolates of *Pedilanthus* leaf curl virus (PeLCV: LT795118, LT795117) on *Brassica rapa* from Pakistan and 90-91% sequence identities with other *begomovirus* isolates.

During Genomatix DiAlign analysis of coat protein gene of (KY511140) with the respective sequences of selected *begomoviruses*, the virus isolate under study showed highest 93-97% similarities at nucleotide (nt) level and 95-97% similarity at amino acid (aa) level with Tomato leaf curl Kerala virus (ToLCKeV) on *Solanum lycopersicum* from India and Pakistan (GQ924760, JF496657). The isolate also shared 84-91% at nt level and 87-96% similarties with aa level with other selected *begomoviruses*: PeLCV, ChLCV, ChLCMV, ToLCKV, CheToLCV, ToLCV (Table 1).

Table 1. *Sequence identities of virus isolate of Malva parviflora (KY511140) with other selected begomovirus isoaltes at nucleotide (nt) and their at amino acid (aa) levels based on genomatix DiAlign programme. (Abbreviation of virus name: Tamato leaf Kerala virus; PeLCV: Pedilanthus leaf curl virus; ChLCV: Chilli leaf curl virus; chLCMV: Chilli leaf curl Multan virus; ToLCKV: Tamato leaf curl Karnataka virus; CheToLCV: Cherry Tamato leaf cuel virus; ToLCV: Tamato leaf cuel virus).*

Accessions	Virus	Host	Country	%Identity Nucleotide	%Identity Amino Acid
KF551575	ToLCKeV	*Solanum lycopersicum*	India	97	98
EU910140	ToLCKeV	*Solanum lycopersicum*	India	96	98
EU910141	ToLCKeV	*Solanum lycopersicum*	India	95	96
LT556075	ToLCKeV	*Solanum lycopersicum*	Pakistan	95	96
KX671963	ToLCKeV	*Brassica rapa*	Pakistan	94	95
LN886521	ToLCKeV	*Solanum lycopersicum*	Pakistan	93	96
KY216063	ToLCKeV	*Solanum lycopersicum*	India	93	97
LT795118	PeLCV	*Brassica rapa*	Pakistan	91	96
LT795117	PeLCV	*Brassica rapa*	Pakistan	91	96
KP195266	ChLCMV	*Solanum lycopersicum*	India	88	95
KU760802	ToLCV	*Solanum melongena*	India	87	94
KX246859	ToLCKaV	*Cajanus cajan*	India	87	91
LN906594	CheToLCV	*Parthenium hysterophorus*	Pakistan	87	90
LN906593	CheToLCV	*Parthenium hysterophorus*	Pakistan	86	87
KX831454	ToLCV	*Ocimum basilicum*	India	86	91
KP195261	ToLCKaV	*Solanum lycopersicum*	India	86	89
AF336806	ChLCV	*Capsicum annuum*	Pakistan	84	94
LN886660	ChLCV	*Capsicum annuum*	Pakistan	84	93

During phylogenetic analysis of virus isolate under study (KY511140) with the other *begomovirus* isolates was done using the Molecular Evolutionary Genetics Analysis Tool (MEGA v.7.1) with 1000 replicates of bootstrapping, and a dendrogram was generated by the neighbour-joining method and viewed using the NJ plot program.

The virus isolate (KY511140) shared close phylogenetic relationships with the isolate of Tomato leaf curl Kerala virus (ToLCKeV: KF551575, EU910140, EU910141, LT556075, LN886521, KY216063) and shared distinct relationships with the isolates of *Pedilanthus* leaf curl virus (PeLCV: LT795118 and LT795117); Chilli leaf curl virus (ChLCV: AF336806, LN886660) on chilli from Pakistan, Chilli leaf curl Multan virus (ChLCMV: KP195266) from tomato from India, Tomato leaf curl Karnataka virus (ToLCKaV: KP195261, KX246859) on *Solanum lycopersicum* and *Cajanus cajan* from India; Cherry tomato leaf curl virus (CheToLCV: LN906594, LN906593) on *Parthenium hysterophorus* from Pakistan and Tomato leaf curl virus (ToLCV: KX831454, KU760802) on *Ocimum basilicum* and *Solanum melongena* from India (Figure 3).

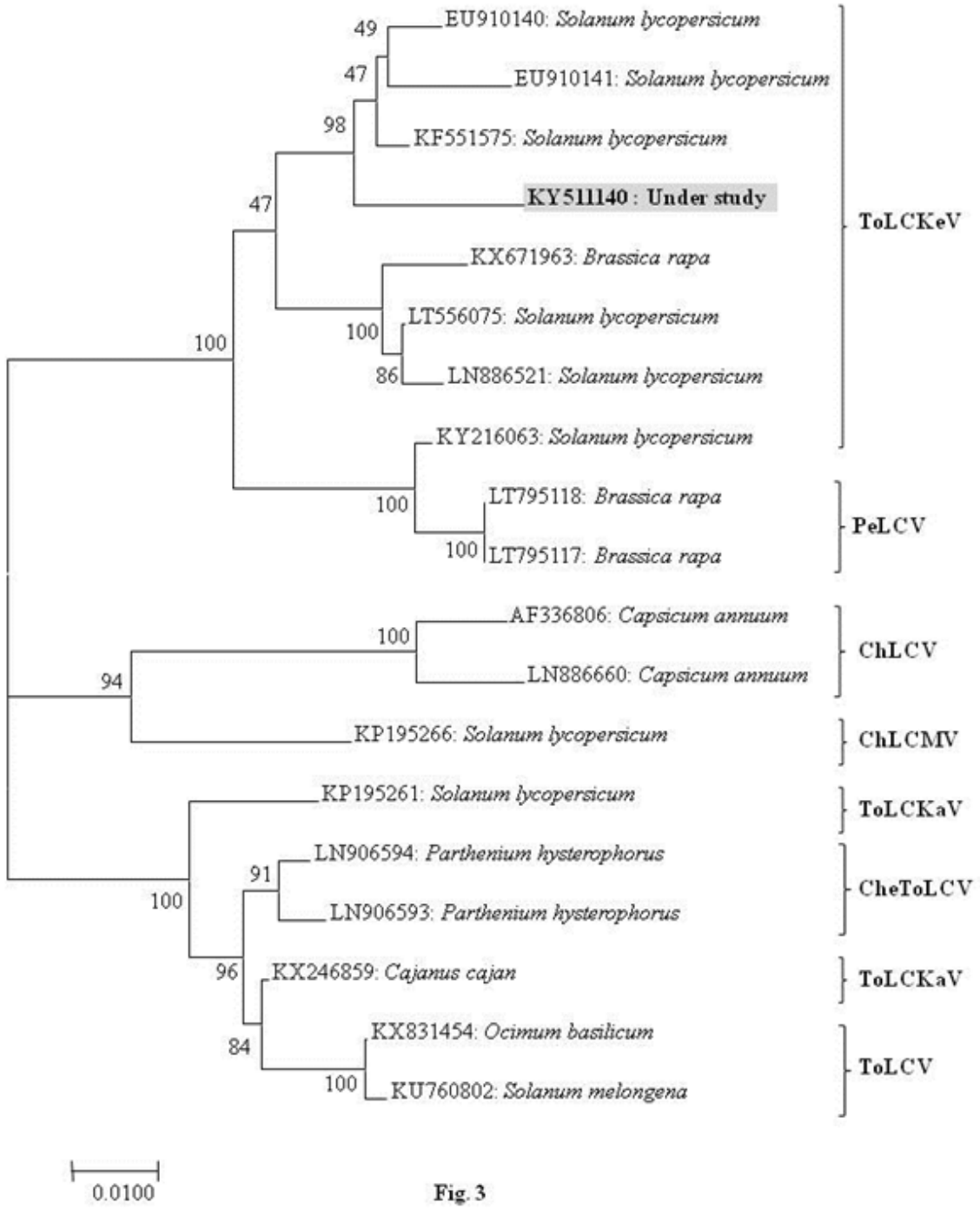

Fig. 3

Figure 3. *Phylogenetic relationships of begomovirus under study (KY511140) with Tamato leaf curl Kerala virus (ToLCKeV), Pedilanthus leaf curl virus (PeLCV), Chilli leaf curl virus(ChLCV), Chilli leaf curl Multan virus(chLCMV),* Tomato leaf curl Karnataka virus(*ToLCKaV*), *Cherry Tamato leaf cuel virus(CheToLCV),Tamato leaf curl virus(ToLCV) selected based on BLASTn analysis was determined by NJ method within MEGA v7.1 program with 1000 bootstrap replicates. Tamato leaf curl Kerala virus (ToLCKeV) strain showing close relationships with isolate under study (KY5411140) (highlighted with gray colour) and showed distinct relationships with other begomovirus strains.*

On the basis of highest sequence identity and the close phylogenetic relationships of the virus isolate of *M. parviflora* with the corresponding sequences of various *begomovirus* isolates reported worldwide, the virus associated with yellow vein net disease of of *M. parviflora* was identified as a *begomovirus* isolate of Tomato leaf curl Kerala virus from Madhya Pradesh, India.

Discussion

India is a large country with very diverse agro-climatic conditions. On the one hand this diverse agro-climate makes India one of the richest sources of flora and fauna in the world, but on the other hand it creates ideal conditions for plant viruses. Although it has been established that weeds can play an important role in the emergence of plant viral epidemics

affecting crops in different parts of the world [18,19], they are still neglected and only limited work has been carried out to characterize the *begomovirus* complexes associated with different weed species in India.

The occurrence *begomovirus* on weed plants have been reported from India like: Ageratum enation virus (AgEV) on *Cleome gynandra* [20], *Crassocephalum crepidioides* and *Ageratum conyzoides* [21]. A new *begomovirus Rhynchosia* yellow mosaic India virus associated with yellow mosaic disease in *Rhynchosia minima* [22]. *Jatropha gossypifolia* weed plants have been reported from the natural occurrence of Croton yellow vein mosaic virus and Croton yellow vein mosaic betasatellite from India [23] and a new *begomovirus Jatropha* yellow mosaic India virus also have been reported from *J. gossypifolia* [2].

Tomato Leaf Curl Kerala Virus (ToLCKeV), a virus prevalent in the tomato crop of Kerala state of India [24,25] and *Alternanthera sessilis* a new host of ToLCKeV have been reported from Rajasthan state, India [26].

In this study the association of the *begomovirus* with severe yellow vein net disease of *M. parviflora* (weed plant) was detected by PCR from using total DNA extracted from symptomatic leaf samples using coat protein gene specific primers for members of the genus *Begomovirus*, which revealed positive amplification of the expected-size bands (~800 bp). On the basis of positive PCR amplification, sequence analysis and phylogenetic relationships, the virus isolate was identified as a *begomovirus* that is closely related to the isolates of Tomatro leaf curl Kerala virus.

M. parvifolia acts as host for some viruses including Malva veinal necrosis virus is considered as belonging to the potato X virus group [10], Tomato yellow leaf curl virus- Isreal [11], South African Cassava mosaic virus [12], Faba bean necrotic yellow virus [13]). The Squash leaf curl virus from Israel was also found to occur naturally in *M. parviflora* [14] and Squash leaf curl virus-Malva from Jordan Valley [15]. Recently, Hollyhock leaf curl virus was identified on *M. parviflora* from Pakistan [16]. However, there are no reports have been published in literature from India about any kind of plant viruses including *begomovirus* on *M. parvifolia* plant.

We report here that the natural occurrence of a *begomovirus* associated with yellow vein net disease of *M. parviflora* identified as a new host of *begomovirus* isolate of Tomato leaf curl Kerala virus from Madhya Pradesh, India. Because *M. parviflora* grows as a weed in India and abroad near the agricultural fields, so that the associated Tomato leaf curl Kerala virus is a serious threat to other commercially important crops and may contribute to the epidemiology of Tomato leaf curl Kerala virus diseases in India.

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