INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 1, p. 266-270, 2018

RESEARCH PAPER OPEN ACCESS

Detection and molecular characterization of begomoviruses infecting chilli pepper

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Key words: Geminivirdae, Sequence demarcation tool, Whitefly-transmitted viruses.

http://dx.doi.org/10.12692/ijb/13.1.266-270 Article published on July 30, 2018

Abstract

Begomovirus is highest economic impact virus on vegetables production in Pakistan. Severe leaf curl disease was noticed on chilli pepper plants growing in agriculture fields in Talagang (Punjab Province, Pakistan). Symptomatic plants exhibited leaf curl, yellow vein and stunted growth. Whitefly transmitted geminiviruses were initially detected by TAS-ELISA using panel of ten monoclonal antibodies. Monoclonal antibodies indicated the presence of begomoviruses. Total DNA was extracted from TAS-ELISA positive samples and PCR was carried out using begomovirus degenerate primers. Sequence demarcation tool (SDT) and phylogenetic anaylsis indicated diversity of begomoviruses infecting chilli pepper. Three begomoviruses are present in chilli pepper. Three identified begomoviruses clusters with *Croton yellow vein mosaic virus* {CYVMV; MG587927}, *Tobacco curly shoot virus* {TbCSV; MG587925} and *Tomato leaf curl karnatka virus* {TLCKV; MG587926} in phylogenetic tree. Results provide serological and molecular evidence that tomato and weeds infecting begomoviruses causes yield loss in the study area in chilli pepper.

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Introduction

The family, Geminiviridae comprises plant viruses with circular, single-stranded (ss) DNA genomes. The genome of monopartite viruses or two genomic components of bipartite viruses are encapsidated in a twinned, isometric particle about 18 x 30 nm in size. Geminiviruses that infect agricultural crops are responsible for substantial economic losses, worldwide (Valverde *et al.,* 2012; Kenyon *et al.,* 2014). The family contains the seven genera, *Becurtovirus, Begomovirus, Curtovirus, Ergrovirus, Mastravirus, Topocuvirus and Turncurtovirus* which differ from each other on the basis of genome organization, host range and insect vector [http://ictv.org/ssdna.viruses/geminivirdae]. The largest and most economically important is the genus, Begomovirus, which are transmitted by the whitefly vector *Bemisia tabaci* (Genn.) in a circulative, persistent manner (Argusello-Astorga *et al.*, 1994; Shih *et al.,* 2003; Brown, 2010).

The number of begomoviruses identified from different plant species has rapidly increased in (Zaidi *et al.*, 2015) leading to the recognition of more than 288 species (Kenyon *et al.,* 2014). Mutation, recombination, pseudo-recombination are major driving forces in the emergence of variants of begomoviruses (Ranjan *et al.*, 2013). The genome of begomoviruses consists of either two genomic components.

The begomoviruses having a bipartite genome arrangements have a DNA-A and DNA-B component, whereas, those with a monopartite genome arrangement have a single DNA-A molecule. In general, arrangements encodes all essential genes, most of which have homologous functions or meet analogous functional requirements. Thus far, most monopartite viruses, particularly those that evolved in Asia and Africa, are associated with one or more satellite molecules that contribute in different ways to pathogenicity, albeit, their contributions to the infection cycle are not entirely understood (Brown *et al.*, 1995; Zhou, 2013). To assess begomovirus diversity and emergence of new begomovirus species present study was carried out.

Materials and methods

The youngest leaves from chilli pepper plants showing typical leaf curl symptoms (upward leaf curling, stunted growth) were collected from vegetable growing fields in Talagang. Leaf samples were collected from eight symptomatic plants and placed in plastic bag and carried to plant virology lab for DNA extraction.

Symptomatic samples were tested by triple antibody sandwich enzyme linked immune sorbent assay (TAS-ELISA) as described by Harrison *et al.*, 1997. Ten monoclonal antibodies, *African cassava mosaic virus* (ACMV), (number SCR 17, 18, 20, 23), *Indian cassava mosaic virus* (ICMV), (SCR 54, 55, 56) and *Okra leaf curl virus* (OLCV), (SCR 102,104,106) were used for epitope profiling (Thomas *et al.*, 1986; Swanson and Harrison,1993).

Total DNA was extracted from eight infected chilli pepper plants by CTAB method as described by (Doyle and Doyle, 1990). The DNA pellet was re-suspended in 100µl of double distilled water. The begomovirus genome was amplified by PCR amplificationusing two oligonucleotide primers for ge miniviruses, pAL1v1978 (GCATCTGCAGGCCCACAT(Y)GTCTT(Y)CCNGT) and pAR1c496 (AATACTGCAG GGCTT(Y)CTRTACATRGG) (Rojas *et al.*, 1993). Amplification of begomoviruses genome was carried out in 50 µl of reaction mixture containing 1µl of template DNA (50 ng), dNTPs (10 mM), Taq buffer and Taqpolymerase. PCR was carried out in thermacycler (thermo fisher) with conditions: initial denaturation at 94°C for 1 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. An expected 1.4bps amplified product was visualized on 0.75% agarose gel. The PCR amplification products were cleaned using wizard gel and PCR cleanup kit (Promega) and send to MACROGEN Korea for bidirectional sequencing. Sequence obtained in FASTA format

were edited and anaylsed in Bioedit and Basic local alignment search tool (BLAST) (Altschul *et al.,* 1990).

Results and discussion

Chilli pepper leaves from symptomatic plants from Talagang (Punjab Province, Pakistan) exhibited severe leaf curling, vein thicking, stunted growth as shown in figure 1.

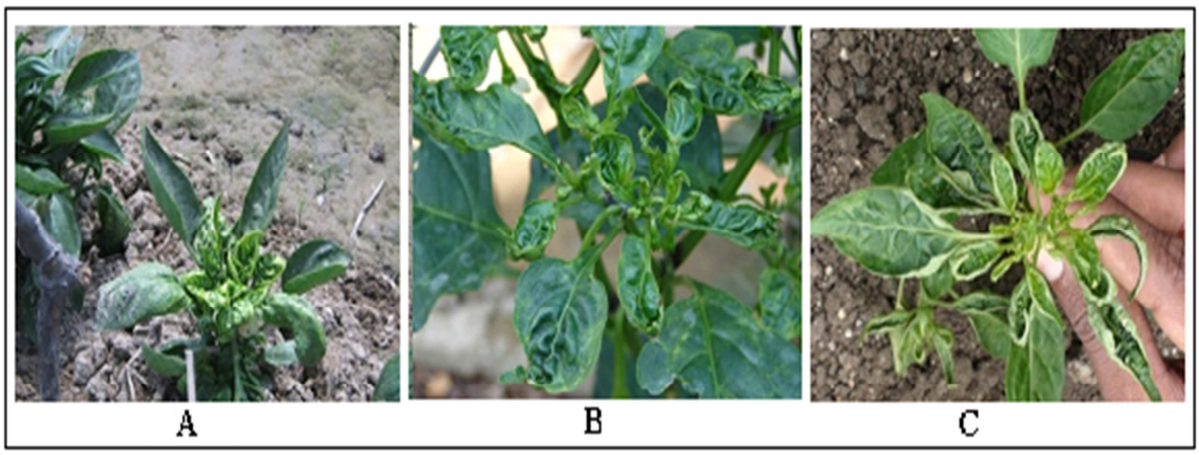

Fig.1. Leaf curl symptoms on chilli pepper plants caused by begomoviruses (A) *Croton yellow vein mosaic virus,* (B) *Tobacco curly shoot virus* and (C) *Tomato leaf curl karnatka virus.*

Serological results showed great diversity of begomoviruses as all monoclonal antibodies reacted from moderate to high level intensity. For molecular detection and identification of begomoviruses associated with leaf curl disease, degenerate primer pair for begomoviruses was used. An expected 1.4bp PCR product obtained from three samples, which was visualized on 0.75% agarose gel.

Two PCR gel cleaned product for each (three) sample were sequenced in both orientation and consensus sequence data were combined to partial DNA genome. Sequence data obtained were analyzed by Basic Alignment Research Tool (BLAST) to check nucleotide sequence identity with all available databases using NCBI-BLAST server (www.ncbi.nlm.nij.gov). Sequence obtained includes a part of replication associated gene, common region and C4 gene. Based on initial BLAST analysis MG587925 showed similarity with *Tobacco curly shoot virus*, MG587927 with *Croton yellow vein virus*, MG587926 with *Tomato leaf curl Karnataka virus* respectively.

Based on highest shared nucleotide identity (SDT) (Muhire *et al.*, 2014) of partial DNA-A genome (MG587925) showed 92% nucleotide sequence identity with *Tobacco curly shoot virus,* (MG587927) isolate sequence was most closely (99.7%) related to *Croton yellow vein mosaic virus*. In addition, MG587926 isolate shared 97% similarity to *Tomato leaf curl Karnataka virus*.

The nucleotide sequences of begomoviruses obtained here were aligned with corresponding nucleotide sequence retrieved from Gen Bank. From alignment data phylogenetic tree was constructed using neighbor-joining method implemented in MEGA V. 6.0 (Tamura *et al.*, 2013) as shown in figure 2. Aforementioned chilli pepper isolates group with corresponding begomovirus in phylogenetic tree as shown in figure. 2.

TbCSV was previously identified in tomato grown in the fields of India (Shilpi *et al.*, 2015). In China TbCSV first detected in pepper, later on occurred in variety of host plants including ageratum (AJ971266, HG003650), *Alternanthera philoxeroides* (GU 199583), *Mirabilis jalapa* (GU199584) (Qiung *et al.*,

2010). TbCSV showed increased virulence with the association of non-cognate betasatellite. Emergence of new begomoviruses has raised serious concern. CYVMV from *C bonplandianum* was transmitted to many vegetables, ornamentals, tobacco varieties and weeds (Pramesh *et al*., 2013). Weeds act as reservoir of begomoviruses and important source of recombination as multiple infection is evident (Mubin *et al*., 2012). ToLCKV is reported from *Glycine max* from Lucknow with high disease incidence of 80%, later on it appeared in economically important chilli pepper crop in Pakistan. The presence of three different begomovirus isolates in single field grown chilli pepper is an example of mixed infection of begomovirus. Decline in chilli pepper production has been attributed to mix infection of begomoviruses.

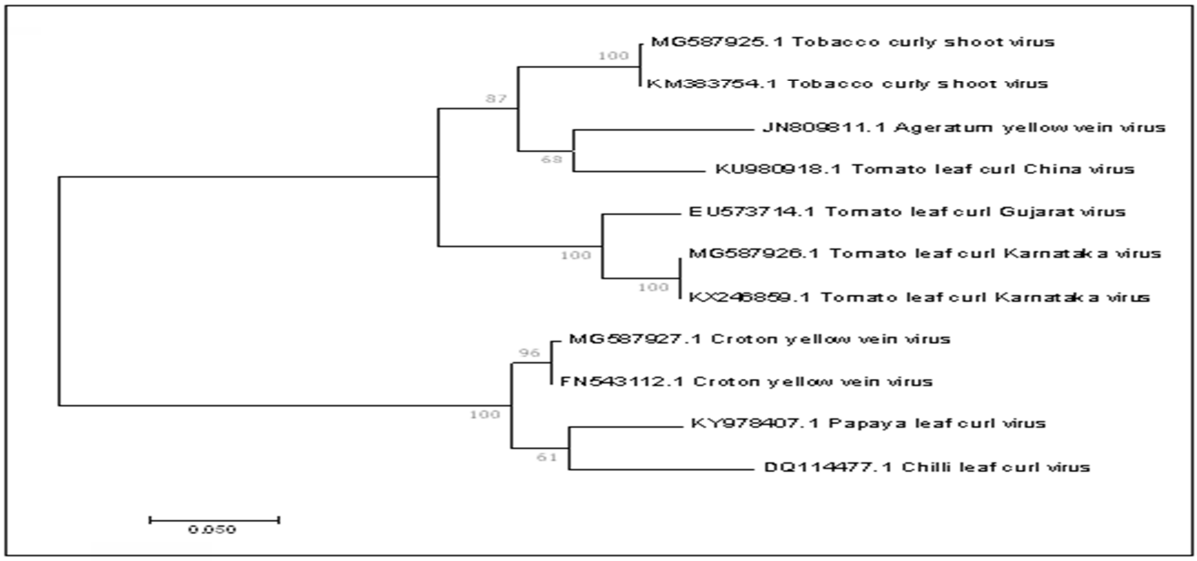

Fig.2. Phylogenetic analysis (Neighbor Joining, 70% bootstrap) implemented in MEGA7 of the begomoviruses from chilli plants in Pakistan, indicating it is most closely related to previously described isolates of begomoviruses.

Acknowledgement

First author extend her thanks to Plant virology lab, CDRI, National Agriculture Research centre Islamabad for providing all necessary research facilities.

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