INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 4, p. 302-312, 2018

RESEARCH PAPER OPEN ACCESS

An insight into genetic variability and host response of Pakistani isolate of *Chilli veinal mottle virus* (ChiVMV) infecting chilli pepper

Tazeem Riaz, Muhammad Ashfaq*, Aamir Humayun Malik, Tariq Mukhtar, Ishfaq Ahmed Hafiz

Department of Plant Pathology, PMAS Arid Agriculture University, Rawalpindi, Pakistan.
Department of Horticulture, PMAS Arid Agriculture University, Rawalpindi, Pakistan.
Biotechnology lab, CAB International, Rawalpindi, Pakistan.

Key words: *Capsicum annum, Chilli veinal mottle virus,* Phylogenetic analysis.

http://dx.doi.org/10.12692/ijb/12.4.302-312 Article published on April 28, 2018

Abstract

Chilli veinal mottle virus (ChiVMV, genus *Potyvirus*) is a wide spread and destructive plant virus that causes substantial economic losses in chilli crop. Although ChiVMV has been reported in Pakistan for more than 22 years, there is little information about the genetic variability of the isolates found in the country. This present study elucidates the genetic variability of a Pakistani Chi VMV isolate ATIPK and its response against different host species. For host response analysis, plant species from *Solanaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae* and *Amarantheaceae* were mechanically inoculated and results revealed that the host range of ATIPK confined to *Solanaceae* family. The 3' genomic end of ChiVMV isolate AITPK containing 96 bases of 3' end of NIb gene, full length CP gene (864) and 23 bases from the 3'UTR. The sequence of ChiVMV (ATIPK) isolate was deposited to Gen Bank with accession No. KJ472764. A pairwise comparison of ATIPK CP sequence with selected 22 sequences revealed the nucleotide identity ranged from 88.4%-86.8% and amino acid identity ranged from 91.8%-89%. Maximum and minimum nucleotide identity was matched with isolate DQ854956 and DQ854964 from Thailand and India respectively. Conserved region of Potyviruses and aphid transmitibility DAG triplet was present in ATIPK isolate at 6-8th amino acid position at the N terminal of CP gene. The information of CP gene sequence and recombination analysis generated in this research will be helpful in development of resistance chilli against ChiVMV in future.

* **Corresponding Author:** Muhammad Ashfaq ✉ mashfaq1642@gmail.com

Introduction

Chilli veinal mottle virus (ChiVMV) is one of the devastate potyviruses infecting chilli pepper around the world including Pakistan (Ahmad *et al.,* 2017).This virus was first time reported from Malaysia on the basis of symptoms and serology by Ong (1979 and 1980) and named as *Chilli veinal mottle virus*. In Pakistan, ChiVMV was first time reported in 1995 from chilli crop (Hameed *et al.,* 1995). Likewise all other potyviruses, ChiVMV has a +ssRNA genome of about 1kb nucleotides enclosed in elongated and flexuous rod shaped particles of 750 nm in length and 12 nm in diameter (Shukla *et al.,* 1994). The genome proteolitically generate 11 functional proteins after cleavage (Adams *et al.,* 2005; Chung *et al.,* 2008). ChiVMV efficiently transmitted in chilli and other solanacious crops through aphids in non-persistant manner (Raccah *et al.,* 1985) and other sources of transmission are the grafting and mechanical inoculation. ChiVMV produce distinctive symptoms of dark green vein-bending and leaf mottling in *C. annuum*. These symptoms are visible and obvious on smaller and younger leaves. Host range of ChiVMV is narrow and confined to solanacious family (Prakash *et al.,* 2002).

The coat protein is best marker for investigating the closely related isolates of potyviruses, as it is the only gene product which shares little sequence identity with the corresponding protein of other virus groups (Berger *et al.,* 2000). The coat protein is involved in the encapsidation of viral RNA, vector transmission (Shukla *et al.,* 1991; Urcuqui-Inchima *et al.,* 2001), the regulation of viral RNA amplification and cell-to-cell movement and systemic movement (Urcuqui Inchima *et al.,* 2001). In this manuscript, reaction of different hosts to ChiVMV infection and the molecular characters of a *capsicum annum* infecting ChiVMV isolate (ATIPK) is described.

Materials and methods

Virus source and identification

The leaves of naturally infected chilli plants showing characteristic symptoms of *ChiVMV* such as, mottling, reduction in leaf and fruit size and dark green leaf banding were collected randomly from farmer's fields located in Islamabad, Pakistan during August, 2012. Symptomatic leaf samples (100 mg) were crushed in extraction buffer and screened for the presence of ChiVMV through Double Antibody Sandwich ELISA using polyclonal antisera of ChiVMV (LOEWE Biochemical, Germany. Cat. No.07185) according to manufacturer's instructions.Absorbance values were determined at (405 nm) with an ELISA Reader.

Virus propagation and host range determination

Crude leaf sap of one gram ELISA positive samples crushed in 0.01 M sodium phosphate buffer (pH 7) and mechanically inoculated on host plants pre-dusted with 600 mesh carborundum. The *Capsicum annum* cv. Loungi was mechanically inoculated with ChiVMV for propagation and constant availability of virus source for biological and molecular studies. For host range study, the virus was mechanically inoculated in range of ten plant species belonging to *Solanaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae* and *Amarantheaceae* families with five replications of each specie (Table 1). All inoculated plants were grown in an insect free glasshouse. The infection of ChiVMV in host plants was confirmed by DAS-ELISA (Clark and Adam, 1977) after 20 days of inoculation.

RNA Isolation, RT PCR and Sequencing

Total plant RNA isolated from inoculated leaf tissues of *Capsicum annum* (cv. Loungi) by trizole reagent method (Life Technologies, Carlsbad, CA, USA) and used to synthesize cDNA (M-MLV first Strand cDNA Synthesis Kit, Life Technologies, Carlsbad, CA, USA) by using oligo (dT) as reverse primer(Tsai *et al.*, 2008).CP gene was amplified by using primer pair CVMV1037/ oligo (dT) (Hiskias, 1998). PCR was executed in 50 µL reaction mixture containing, 5 µL of 10X PCR buffer, 1 µL of 10 mMdNTP's, 3µL of 25 mM $MgCl_2$, 1 µL of 20 pM (forward primer), 1 µL of 20 pM (reverse primer), 0.4 µL (500 U) of Taq polymerase enzyme, 34.6 µL of nuclease free water and 4 µL of cDNA template. The amplification reaction was carried out at 94 °C for 5 minutes followed by 35 cycles of 1 minutes at 94 °C, 1 minutes

at 50 °C and 2 minutes at 72 °C with final extension step at 72 °C for 5 minutes. Amplified fragments were visualized by electrophoresis in 1.0 % (w/v) agarose gel stained with ethidium bromide @100 µg/mL. Purified PCR product (Purified by Pure Link® PCR Purification Kit by Life Technologies, Carlsbad, CA, USA) was ligated into the pTZ57R/T (Thermo Scientific, EU. Ins TAclone PCR Cloning Kit) vector through heat shock transformation in *E.* coli strain DH5α. Plasmid DNA was purified (Thermo Scientific, EU. Gene JET Plasmid Miniprep Kit) according to manufacturer instructions and positive clones were sequenced.

Phylogenetic Analysis

Sequence comparison and phylogenetic analysis was performed on whole CP coding region. Sequence of selected clone was aligned using Clustal W programme and compared with 22 highly matched ChiVMV sequences available in data base using BLASTn application ("http:/www.ncbi.nlm.nih.gov/BLAST/blastn").

Identities matrix for nt and a were calculated using the "Sequence Identity Matrix" option in Bio Edit program version 7.25 (http://www.mbio.ncsu.edu/BioEdit/bioedit.htmL).

The phylogenetic trees was constructed by the maximum likelihood method from Clustal W in MEGA version 6.0 program (Tamura *et al.,* 2013) and bootstrap analysis was conducted in 1000 replications.

Results

Confirmation and propogation

Field collected leaves sample that showed the highest absorbance value in DAS ELISA test were selected for the propagation on *capsicum annum* cv Loungi and named as ChiVMV isolate ATIPK.

Table 1. Reaction of different host species against ChiVMV isolate ATIPK after mechanical inoculation.

S. #	Host plant	Plant family	No. of plants tested	Symptoms	ELISA OD Value
1	*C. annum* cv. Sanum and cv. Loungi	*Solanaceae*	5	Severe mottling, mosaic, vein clearing and vein banding	2.98
2	*N. tabaccum*	*Solanaceae*	5	Severe mottling, mosaic and leaf curling	3.01
3	*Datura metal*	*Solanaceae*	5	Mottling, vein clearing and rat tailed	2.99
4	*S. lycopersicum*	*Solanaceae*	5	Mottling, mosaic and leaf curling	3.1
5	*S.nigrum*	*Solanaceae*	5	Mottling and mosaic	2.71
6	*C. amarinticular*	*Chenopodiaceae*	5	Nil	-ve
7	*Cucumisstavis*	*Cucurbitaceae*	5	Nil	-ve
8	*Vignamungo*	*Fabaceae*	5	Nil	-ve
9	*Vignaradiata*	*Fabaceae*	5	Nil	-ve
10	*S. oleracea*	*Amarantheaceae*	5	Nil	-ve

OD value for each specie showed the mean value of five replications.

Host range determination

The 14 days old infected leaf samples from propagative host were used for the host range determination. Only 5 plant species showed positive reaction against ChiVMV isolate ATIPK (Table 1). The inoculated leaves of the positively reacted plant species exhibited symptoms 14-18dpi. The symptomatic plants of chilli varityviz; cv. Sanam clearly showed symptoms of leaf banding, dark green vein banding, mosaic, mottling and reduction in leaf

and fruit size (Fig.1). *Nicotiana tabaccum* produced mottling, mosaic and leaf curling after inoculation. *Datura metal* displayed mottling and vein clearing symptom. *Solanum lycopersicum* and *Solanum nigrum* showed the symptoms of mosaic, mottling and leaf curling. The infection of ChiVMV was confirmed by DAS ELISA after 20 days of inoculation. Five plant species reacted positively in DAS-ELISA with ChiVMV antiserum (Table 1). The rest of five plants did not become infected upon repeated inoculations observed till 30 dpi; no latent infection was detected in any plant through DAS ELISA. Un-inoculated control plants did not give any symptoms and were also ELISA negative.

Table 2. Twenty two isolates of *Chilli veinal mottle virus* (ChiVMV) used in phylogenetic study.

Country	Isolate name	Accession number	Reference
China	YN75	HQ218936	Ding *et al.,* 2011
Indonesia	Cikabayan2	DQ854960	Tsai *et al.,*2008
Taiwan	ChiVMV-VN/C3	DQ925442	Ha *et al.,* 2008
Thailand	SKh5	DQ854959	Tsai *et al.,* 2008
Thailand	K37	DQ854956	Tsai *et al.,* 2008
Thailand	CM1	DQ854953	Tsai *et al.,* 2008
Thailand	BP	DQ854954	Tsai *et al.,* 2008
Thailand	SRt8	DQ854958	Tsai *et al.,* 2008
Veitnam	ChiVMV-VN/C5	DQ925444	Ha *et al.,* 2008
India	S7	EF213700	Unpublished
India	Be21	EF213684	Unpublished
India	CHL40	EF213681	Unpublished
India	CH34	EF213679	Unpublished
India	DCV3	DQ854965	Unpublished
India	Be22	EF213685	Unpublished
India	PM1	EF213703	Unpublished
India	BCV1	DQ854962	Unpublished
India	BeCV1	DQ854963	Tsai *et al.,*2008
India	CCV3	DQ854964	Tsai *et al.,*2008
Indonesia	2	AB703256	Unpublished
India	D10	EF221615	Unpublished
Indonesia	Pataruman	DQ854961	Tsai *et al.,* 2008

RT-PCR and phylogenetic analysis

Presence of ChiVMV was confirmed by using CVMV1037 Pol as upstream and oligo (dT) as downstream primer. Fragments of approximately 1.2 kb were amplified from each ChiVMV positive sample (Fig. 2). The amplicons were cloned into pTZ57R/T vector (Fig. 3) and sequenced in both orientations. In BLASTn analysis the sequences of all clones were identified as 983 nucleotides of 3' end of ChiVMV, including 96 bases of Nib gene and 864 nucleotides of CP gene (deducing 287 amino acids) and 23 nucleotides of 3′ untranslated region with a poly adenylated tail. The sequences of three isolates were identical, so the sequences of one isolate was submitted to Gen Bank under accession number KJ472764.

For phylogenetic analysis, total of 22 best matched nucleotide sequences from different geographically locations were picked from NCBI and aligned (Table 2).The phylogenetic analysis based on CP gene nucleotide sequences of ChiVMV isolate ATIPK

showed that the Pakistani isolate was clustered into two divergent clades (I and II).Clade I contained the 21 isolates from India, Taiwan, Thailand and Indonesia while clade II contained only two isolates, one from Pakistan (KJ472764) and other from China (Fig. 4).Nucleotide identity ranged from 88.4%-86.8%, highest nucleotide identity was observed as 88.4% with an isolate from Thailand (DQ854956) and lowest nucleotide identity was observed as 86.8% with an isolate from India (DQ854964). While amino acid identity ranged from 91.8% -89.3%. Highest value of amino acid identity (92.7%) was observed with 3 isolates from India (EF213679, EF213681 and EF213703) and lowest amino acid identity was 89.3% for an isolate from Indonesia (AB703256).

Fig.1. Symptoms development on host species after mechanical inoculation with ChiVMV isolate ATIPK. (A and B) Chilli varities (Sanam and Loungi) showing symptoms of mosaic, mottling, leaf distortion, vein clearing and reduced sized leaves (C) *Nicotiana tobaccum* showing mosaic, mottling and vein clearing (D) *Datura metal* showing mottling and vein clearing (E) *Solanum lycopersicum* plants howing symptoms of mosaic, mottling, leaf distortion, vein clearing and reduced sized leaves (F) *Solanum nigrum* plants showing symptoms of mottling, leaf distortion and reduced sized leaves.

The cleavage site between NIb and CP gene was Q/A, DAG motif and potyviruses conserved region was also present in ChiVMV isolate ATIPK (Fig. 5).

Discussion

Host response against ChiVMV isolate ATIPK Chilli veinal mottle virus (ChiVMV) is the most devastating threat to the chilli crop in Pakistan. The main objective of present work was to highlight the phylogenetic position and genetic variability of ChiVMV isolate ATIPK among potyviruses found elsewhere in the world. ChiVMV isolate ATIPK was isolated from the naturally infected chilli leaves, the result of host range studies for ChiVMV isolate ATIPK

verified the previous reports by Siriwong *et al.,* (1995) that ChiVMV has narrow host range, confined to the solanaceous family. The present results of the host range studies for ChiVMV isolate ATIPK are inconsistent with the earlier workers (Parakash *et al.,* 2002; Moury*et al.,* 2005) who have also observed systemic sever mottling, mosaic and vein banding on chillivarities, *Nicotiana tabaccum*, *Datura metal* and *Solanum nigram* as by ChiVMV isolate ATIPK. Likewise are the findings of Shah *et al.,* (2008) but contradict in some respect i.e. ChiVMV isolate ATIPK did infect *Solanum lycopersicum* while their isolate did not infect this host. Recently Zhao *et al.,* (2014) and Ahmad *et al.,* (2017) also reported similar results as we observed after inoculation of tomato by using ChiVMV isolate ATIPK, from china and Pakistan respectively.

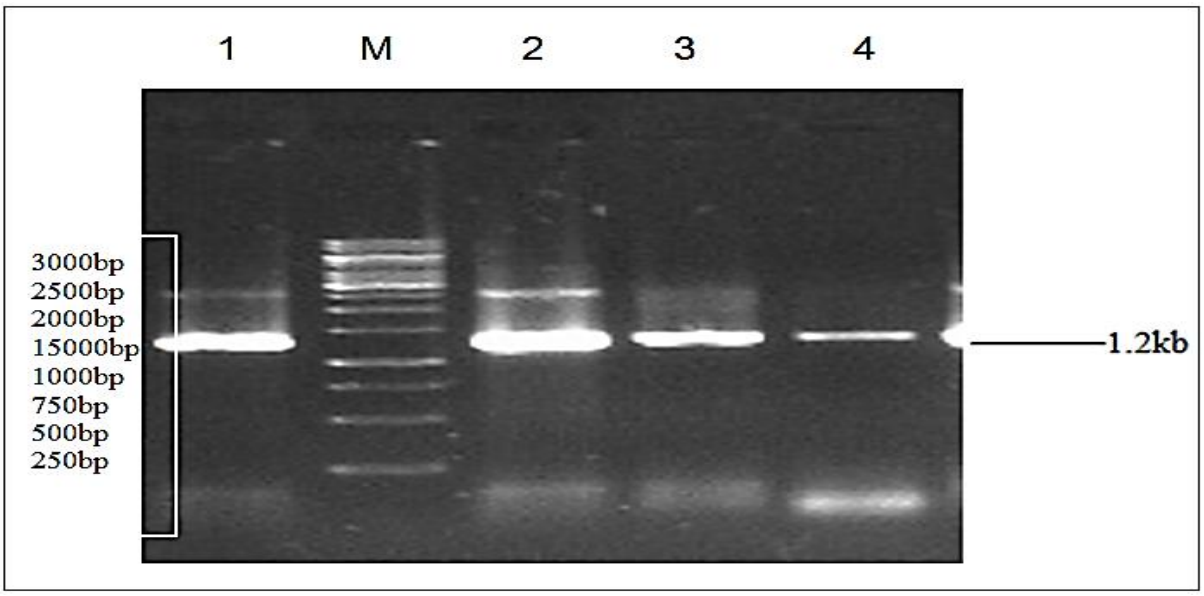

Fig. 2. RT-PCR amplification of CP gene with CVMV1037/oligo(dT). Lane 1, 2, 3 and 4 contains 1.2kb amplified product of NIb and CP gene.

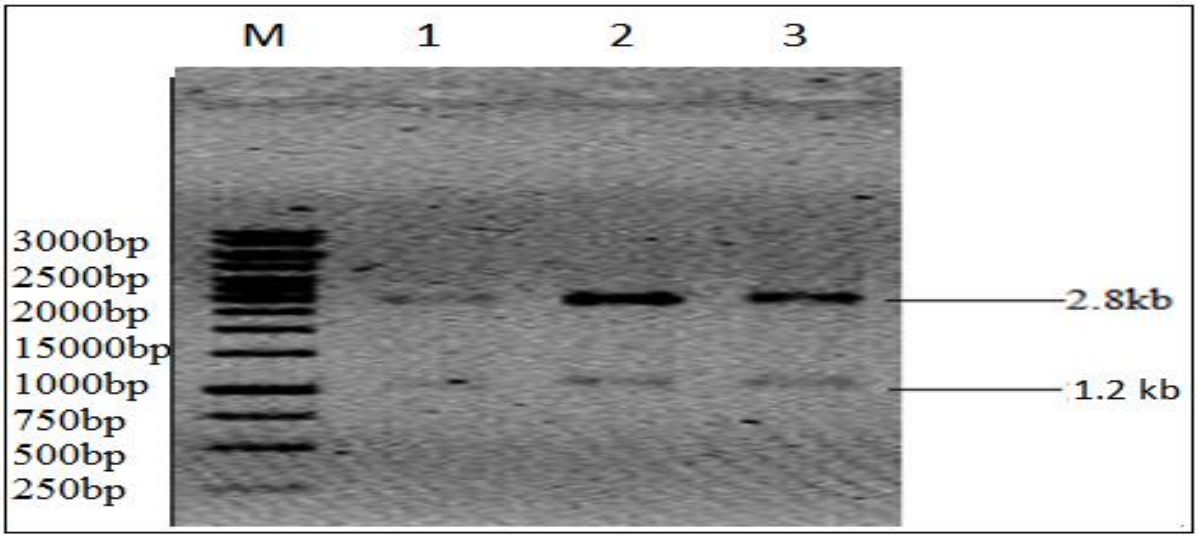

Fig. 3. Clones digested with *EcoR*1 and *Pst1*.

Phytogenetic analysis and genetic variability

Nucleic acid and deduced amino acid analysis of the coat protein gene from potyvirus has been assumed an authoritative tool for taxonomic studies of the genus and strains of this group. The deduced amino acid sequence of the complete CP gene contained 267

residues showing a close relationship to ChiVMV, indicating that it was an isolate of ChiVMV. For potyviruses, DAG motif located near the N-terminal region of CP gene is essential for virus transmission by insect (Aphid) vector (Dombrovsky *et al.,* 2005; Ha *et al.,* 2008; Ding *et al.,* 2011). DAG box was also present in Pakistani ChiVMV isolate that was located at amino acid position of 6-8 residues from the proposed N-terminus of the CP gene. The MVWCIENGTSP conserved region of potyviruses (Ward *et al.,* 1992) was also conserved in CP of Pakistani ATIPK isolate. All the potyviruses have the putative proteolytic cleavage site VYHQ/S between NIb and CP gene (Shukla *et al.,* 1991; Atreya, 1992, Wang *et al.,* 2007) while the analysis of Pakistani isolate of ChiVMV revealed that it had cleavage site Q/A instead of Q/S as reported in all other potyviruses but same cleavage site was also reported between NIb and CP gene of two Indian isolates (BCV1 and DCV3) of ChiVMV by Tsai *et al.* (2008).

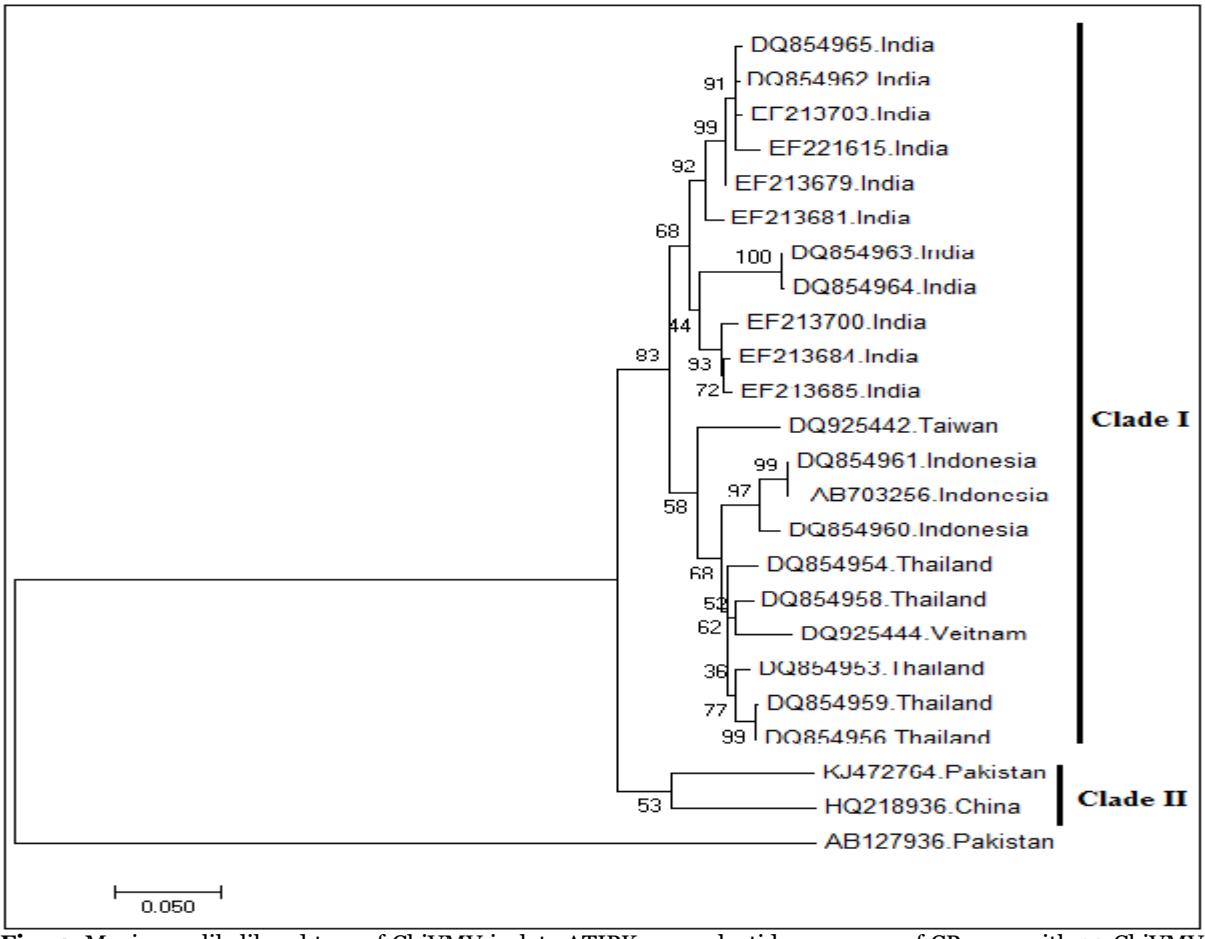

Fig. 4. Maximum likelihood tree of ChiVMV isolate ATIPK on nucleotide sequence of CP gene with 22 ChiVMV isolates from the world. The bootstrap analysis was conducted in 1000 replications. ZYMV (AB127936) sequence is used as out-group.

The CP nucleotides sequence of ChiVMV isolate ATIPK had substitutions of 68 bp at N-terminal region of CP gene. Substitutions were present mostly in first 200 nucleotides out of the total 864 bases. The presence of the separate phylogenetic clade of Pakistani and Chinese isolate indicates a possible source and route for the ChiVMV isolate ATIPK from China in Pakistan,but it is not necessary that any prevailed virus adopt the same entrance way to spread quickly in geographically adjacent areas (Pfosser and Baumann, 2002).

This study has revealed information about the phylogenetic relationship and position of Pakistani ChiVMV isolate ATIPK. The use of CP gene

"conserved region" of Pakistani ChiVMV isolate ATIPK can be helpful to produce the transgenic model plants in future, through PDR approach. PDR approach becoming the method of choice because it attained the effective resistance against many plant viruses. Until now, many PDR lines have been magnificently developed against different potyviruses that exhibit the efficient control (Maki-Valkama *et al.,* 2000; Lines *et al.,* 2002; Faivre-Rampant *et al.,* 2004; Missiou *et al.,* 2004; Bai *et al.,* 2009).

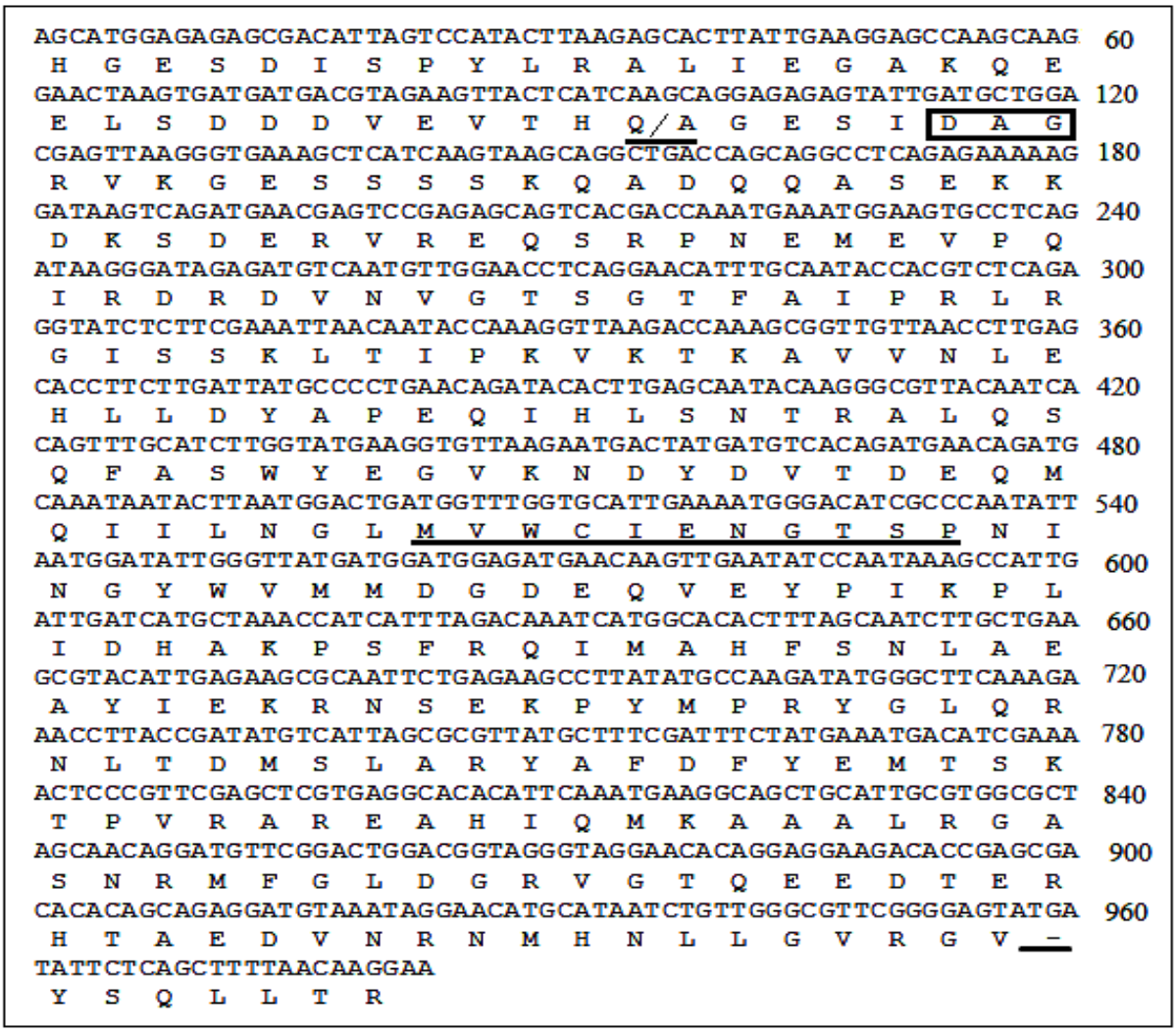

Fig. 5. The nucleotide and deduced amino acid sequence of the cloned 3'-terminal region of CP gene of ChiVMV, isolate ATIPK (Accession No. KJ472764). The cleavage site (Q/A) between NIb and CP, the conserved region of the core of CP of potyviruses and the stop codon are underlined, the DAG motif for aphid transmissibility is boxed.

Acknowledgments

We are thankful to Higher Education Commission of Pakistan for funding this research to the first author (Pin # 106-12055-AV6-133) under Indigenous PhD Fellowships for 5000 Scholars, HEC (Phase-VI).

References

Adams MJ, Antoniw JF, Fauquet CM. 2005. Molecular criteria for genus and species discrimination within the family Potyviridae. Archives of Virology **150,** 459- 479. http://dx.doi.org/10.1007/s00705-004-0440-6

Ahmad A, Ashfaq M, Mukhtar T, Malik SI. 2017. An insight into recombination in CP gene of

tomato infecting Chilli veinal mottle virus isolate from Pakistan. International Journal of Biosciences **11,** 48-54.
http://dx.doi.org/10.12692/ijb/11.4.48-54

Atreya CD. 1992. Application of genome sequence information in potyvirus taxonomy, an overview. Archives of Virology **5,** 17-23.
http://dx.doi.org/10.1007/978-3-7091-6920-9_2

Bai Y, Guo Z, Wang X, Bai D, Zhang W. 2009. Generation of double virus resistant marker-free transgenic potato plants. Progress in Natural Science **19,** 543-548.
http://dx.doi.org/10.1016/j.pnsc.2008.08.005

Berger PH, Barnett OW, Brunt AA, Colinet D, Edwardson JR, Hammond J, Hill JH, Jordan RL, Kashiwazaki S, Makkouk K, Morales FJ, Rybicki E, Spence N. 2000. Virus taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, London, 703-724.

Chung BY, Miller WA, Atkins JF, Firth AE. 2008. An overlapping essential gene in the Potyviridae. Proc. Natl. Acad. Sci. U. S. A **105,** 5897-5902.

Clark MF, Adams AN. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology **34,** 475-83.
http://dx.doi.org/10.1099/0022-1317-34-3-475

Ding M, Yang C, Zhang Z, Jiang ZL, Fang Q, Qin XY, Zhang ZK. 2011. Occurrence of Chilli veinal mottle virus in Nicotiana tabacum in Yunnan, China. Plant Disease **95,** 3572.
http://dx.doi.org/10.1094/PDIS-09-10-0686

Dombrovsky A, Huet H, Chejanovsky N, Raccah B. 2005. Aphid transmission of a potyvirus depends upon suitability of the helper component and N-terminus of the coat protein. Archives of Virology **150,** 287-298.
http://dx.doi.org/10.1007/s00705-004-0407-7

Faivre-Rampant O, Gilroy EM, Hrubikova K, Hein I, Millam S, Loake GL, Birch P, Taylor M, Lacomme C. 2004. Potato virus X-induced gene silencing in leaves and tubers of potato. Plant Physiolology**134,** 1308-1316.
https://doi.org/10.1104/pp.103.037507

Ha C, Revill P, Harding RM, Vu M, Dale JL. 2008. Identification and sequence analysis of potyviruses infecting crops in Vietnam. Archives Virology **153,** 45-60.
http://dx.doi.org/10.1007/s00705-007-1067-1

Hameed S, Khalid S, Ali H, Shah H. 1995. Prevalence of chilli viruses in Pakistan. Fifth National Conference of Plant Sciences., NARC Islamabad, Pakistan. 128 p.

Hiskias Y.1998. Characterization of potyvirus isolates from hot pepper and tomato in Ethiopia. PhD thesis, Institute of Plant Pathology and Plant Protection, Georg-August University, Gottingen, Germany.

Lines RE, Persley D, Dale JL, Drew R, Bateson MF. 2002. Genetically engineered immunity to Papaya ringspot virus in Australian papaya cultivars. Molecular Breeding **10,** 119-129.
http://dx.doi.org/10.1023/A:1020381110181

Maki-Valkama T, Valkonen JPT, Kreuze JF, Pehu E. 2000. Transgenic resistance to PVYO associated with post-transcriptional silencing of the P1 transgene is overcome by PVYN strains that carry highly homologous P1 sequences and recover the transgene expression at infection. Molecular Plant-Microbe Interaction **13,** 366-373.
http://dx.doi.org/10.1094/MPMI.2000.13.4.366

Missiou A, Kalantidis K, Boulta A, Tzortzakaki S, Tabler, Tsagris M. 2004. Generation of

transgenic potato plants highly resistant to Potato virus Y (PVY) through RNA silencing. Molecular Breeding **14,** 185-197. http://dx.doi.org/10.1023/B:MOLB.0000038006.32812.52

Moury B, Palloix A, Caranta C, Gognalons P. 2005. Serological, molecular, and pathotype diversity of Pepper veinal mottle virus and Chilli veinal mottle virus. Phytopathology **95,** 227-32. http://dx.doi.org/10.1094/PHYTO-95-0227

Ong CA, Ting WP. 1977. A review of plant virus diseases in Peninsular Malaysia. Symposium on Virus Diseases of Tropical Crops, Tropical Agriculture Researches Series **10,** 155-164.

Ong CA, Varghese G, Poh TW. 1979. Aetiological investigation on a veinal mottle virus of chili (Capsicum annum L.) newly recorded from Peninsular Malaysia. Malaysian Agriculture Research and Development Intitute, Research Bulletin **7,** 78-88.

Pfosser MF, Baumann H. 2002. Phylogeny and geographical differentiation of Zucchini yellow mosaic virus isolates (Potyviridae) based on molecular analysis of the coat protein and part of the cytoplasmic inclusion protein genes. Archives of Virology **147,** 1599-1609. https://doi.org/10.1007/s00705-002-0839-x

Prakash S, Singh SJ, Singh RK, Upadhyaya PP. 2002. Distribution, incidence and detection of a Potyvirus on chilli from eastern Uttar Pradesh. Indian Phytopathology **55,** 294-298.

Raccah B, Gal-On A, Eastop VF. 1985. The role of flying aphid vectors in the transmission of cucumber mosaic virus and potato virus Y to peppers in Isreal. Annals of Applied Biology **106,** 451-460. http://dx.doi.org/10.1111/j.1744-7348.1985.tb03135.x

Shah H, Yasmin T, Fahim M, Hameed S, Haque MI. 2008. Transmission and host range studies of Pakistani isolates of Chilli veinal mottle virus. Pakistan Journal of Botany **40,** 2669-2681.

Shukla DD, Frenkel MJ, Ward CW. 1991. Structure and function of the potyvirus genome with special reference to the coat protein coding region. Canadian Journal of Plant Pathology **13,** 178-191.

Shukla DD, Ward CW, Brunt AA. 1994. The Potyviridae. CAB International, Wallingford, UK, 516p.

Siriwong P, Kittipakorn K, Ikegami M. 1995. Characterization of Chilli vein-banding mottle virus isolated from pepper in Thailand. Plant Pathology **44,** 718-727. http://dx.doi.org/10.1111/j.1365-3059.1995.tb01696.x

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA 6: Molecular evolutionary gtenetics analysis version 6.0. Molecular Biology Evolution **30,** 2725-2729. http://dx.doi.org/10.1093/molbev/mst197

Tsai WS, Huang YC, Zhang DY, Reddy K, Hidayat SH, Srithongchai W, Green SK, Jan FJ.2008. Molecular characterization of the CP gene and 3UTR of Chilli veinal mottle virus from South and Southeast Asia. Plant Pathology **57,** 408-416. http://dx.doi.org/10.1111/j.1365-3059.2007.01780.x

Urcuqui-Inchima S, Haenni AL, Bernardi F. 2001. Potyvirus proteins, a wealth of functions. Virus Research **74,** 157-175. http://dx.doi.org/10.1016/S0168-1702(01)00220-9

Wang LY, Chen KC, Chen TC, Yeh SD. 2007. Nucleotide sequence analysis of the coat protein genes of two isolates of Sweet potato feathery mottle virus from central Taiwan. Plant Pathology Bulletin **16,** 203-213.

Ward CW, McKern NM, Frankel MJ, Shukla DD.1992. Sequence data as the major criterion of potyvirus classification. Archives of Virology **5,** 283-297.
http://dx.doi.org/10.1007/978-3-7091-6920-9_29

Yang J, Dong JH, Zhang TJ, Wang R, Luo ZP, Zhang ZK. 2013. A new isolate of Chilli veinal mottle virus that infect tobacco in China. Journal of Plant Pathology **95,** 187-190.
http://dx.doi.org/10.4454/JPP.V95I1.006

Zhao FF, Xi DH, Liu J, Deng XG, Lin HH. 2014. First Report of Chilli veinal mottle virus Infecting Tomato (Solanum lycopersicum) in China. Plant Disease **98,** 1589-1589.
https://doi.org/10.1094/PDIS-11-13-1188-PDN