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An insight into recombination in CP gene of tomato infecting *Chilli veinal mottle virus* isolate from Pakistan

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Abstract

Chilli veinal mottle virus (ChiVMV) is one of the significant Potyviruses infecting solanaceous crops. One of the key factors in successful survival and adoptability of an individual is genetic diversity, which is commonly influenced by recombination events. In this manuscript, molecular characterization and recombination analysis based on 3' genomic end of a tomato infecting ChiVMV isolate (AARTPK) is presented. The 3' genomic end of ChiVMV isolate AARTPK containing coat protein (CP) gene (459 bp) and 3' untranslated region (390 bp) was amplified by using degenerate and specific primers in reverse transcription polymerase chain reaction (RT-PCR). In CP gene BLAST analysis, Pakistani isolate (AARTPK) shared 91-93% and 93-96% nucleotide and deduced amino acid sequence identities respectively, with other isolates of ChiVMV. In nucleotide sequence based phylogenetic analysis AARTPK clustered with Chines (YN75, YN-tobacco and Dzh-Qyg) and Italian isolate (74PAV) isolates. The information of CP gene sequence and recombination analysis generated in this research will be helpful in development of resistance tomato against ChiVMV in future.

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the 2nd most important crop after potato in term of consumption and production worldwide including Pakistan (Kamran *et al.,* 2012; Tahir *et al.,* 2012). It is an excellent source of vitamins (A, B and C) (Kothari *et al.,* 2010) and minerals like iron, phosphorous and carotenoids having a high oxygen-radical quenching and scavenging capability (Babalola *et al.,* 2010). Several potyviruses, like *Tobacco etch virus* (TEV), *Potato virus Y* (PVY), *Chilli veinal mottle virus* (ChiVMV) and *Pepper veinal mottle virus* (PVMV) have been previously reported to infect on tomato plants globally (Kothari *et al.,* 2010; Rialch *et al.,* 2015). ChiVMV is one of the lethal viral pathogen with variety of hosts like *Capsicum annuum,* C. *frutescens, Solanum melongena, Datura stramonium*, and *Chenopodium* spp. (Green and Kim, 1991), *Nicotiana* spp. (Yang *et al.,* 2013) *Solanumlycopersicum* (Zhao *et al.,* 2014; Ahmad and Ashfaq, 2017),*Buddleja crispa* (Mehra *et al.,* 2006) causing upto 50% yield losses. The virus is widely distributed in Asia (Tsai *et al.,* 2008) and can cause yield losses upto 50% (Ong *et al.,* 1979). The aphid specie named *Aphis gossypii* (Glov.) is known to transmit ChiVMV to solanaceous crops non-persistently (Ong *et al.,* 1979; Green *et al.,* 1999; Shah *et al.,*2008).Likewise, all other potyviruses the genome of ChiVMV is a single-stranded RNA of 9.7 kb with a 3'-terminal poly(A) tail enclosed in a flexuous coat protein of 765x13 nm in size (Siriwong *et al.,* 1995). The genome encodes a polyprotein of ca. 350 kDa which is cleaved into 10 functional proteins by a virus-encoded proteinases (Siriwong *et al.,* 1995; Anindya *et al.,* 2004).

In successful survival and adoptability of an individual, genetic diversity is of vital importance. In case of RNA viruses, mutations initiated by frequent errors in RNA synthesis (Domingo and Holland, 1994; Drake, 1993; Roossinck, 1997) and the recombination events (Simon and Burjarski, 1994; Aranda *et al.,* 1997) are considered as major causes of genetic variability. Although, in natural populations of plant viruses, the recombination events are rarely observed (Fraile *et al.,* 1997), yetnumerous potyviruses has been investigated for possible recombination (Revers *et al.,* 1996). In this manuscript, the molecular characterization and recombination analysis of a tomato infecting ChiVMV isolate (AARTPK) is described.

Materials and methods

Virus source and maintenance

The isolate AARTPK was selected from samples collected from the research farm of PMAS-Arid Agriculture University Rawalpindi (Ahmad and Ashfaq, 2017) and maintained on healthy tomato plants var. sahil. Crude sap of 1 gram ELISA positive samples homogenized in 10 ml of 0.01 M sodium phosphate buffer (pH 7) was mechanically inoculated on healthy tomato plants pre-dusted with 600 mesh carborundum. The presence of ChiVMV in the inoculated plants was confirmed by DAS-ELISA, after three weeks of inoculation.

RT-PCR and sequence analysis

Total RNA isolated from ELISA positive tomato leaf samples by Trizol reagent (Invitrogen) was used in RT-PCR (using GoTaq® Green Master Mix, Promega) with specific primers CVMVPK-F/R targeting complete CP gene and 3'UTR of ChiVMV genome (Ahmad and Ashfaq, 2017). The amplicons were purified by using QIAquick PCR purification kit (Qiagen) and were sequenced in forward and reverse direction from Macrogen, Korea. The obtained sequences were subjected to BLASTn analysis. Percent nucleotide and amino acid identities were calculated using BioEdit v7.2.6.1. Multiple sequence alignments of nucleotides and deduced amino acid sequences (using the EMBOSS Transeq program, Rice *et al.,* 2000) and phylogenetic analysis (using neighbour joining method and 1000 bootstraps) were performed by using MEGA 6 software (Tamura *et al.,* 2013). Pepper veinal mottle virus (PVMV) was used as a reference out group for rooting the phylogenetic tree. Recombination analysis was carried out by using RDP package (Martin *et al.,* 2010) using the default settings plus the Bonferroni corrected P-value cut-off (a = 0.05).

Recombination breakpoints were considered as significant if supported by four or more methods (RDP, MaxChi, Bootscan, 3SEQ, Lard, GENECONV, Chimaera and Siscan).

Results

Confirmation of Koch's postulates

Tomato plants inoculated with ChiVMV produced mosaic, necrosis and mottling foliar symptoms after two weeks of inoculation. In DAS-ELISA all inoculated plants reacted positively with ChiVMV antiserum.

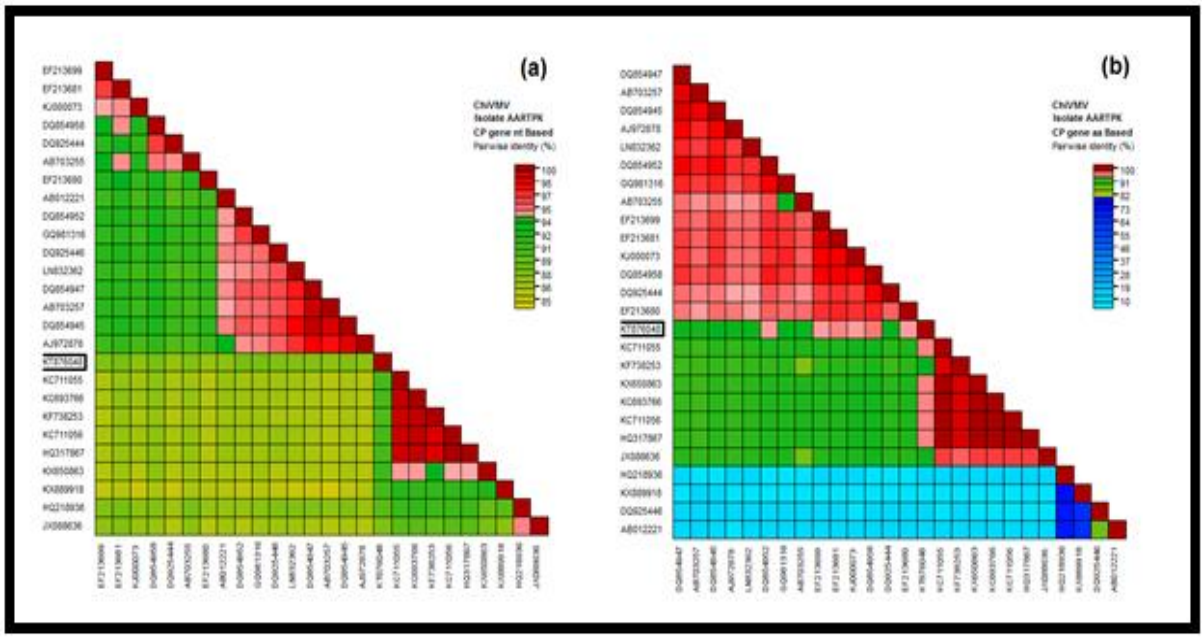

Fig. 1. Percentage (a) nucleotides and (b) amino acids identities of isolate AARTPK with other isolates of ChiVMV.

RT-PCR and sequence analysis

The RT-PCR resulted in the amplification of 1160 bases. The obtained nucleotide sequence of isolate AARTPK was deposited to NCBI (Acc. No. KT876048). The sequence was comprised of1160 nucleotides of 3' end including 860 nucleotides of CP gene (deducing 286 amino acids) and 300 nucleotides of 3′ untranslated region with a poly adenylated tail. The deduced amino acid sequence was inspected for conserved motifs. The putative NIb and CP genes had a cleavage site Q/A between them. Other conserved motifs like DAG, AFDF and QMKAAL were located at 6-8, 222-225 and 242-248 amino acid positions respectively, from N-terminal of CP gene. In CP gene the composition of Adenine, Cytosine, Guanine and Uracil was recorded as 33%, 20%, 23% and 23% respectively. In BLAST analysis the nucleotide sequences of CP gene plus 3′UTR of Pakistani isolate was 82·4 to 90·5% identical to formerly reported ChiVMV isolates. The sequence of CP gene was91-93% and 93-96% at nucleotide (Fig. 1a) and amino acid (Fig. 1b) level respectively with other ChiVMV isolates. Similarly, the nucleotide sequence of 3'untranslated region shared 94.5% identity with formerly reported ChiVMV isolates. In phylogenetic analysis nucleotide sequence of CP gene of AARTPK, clustered with Chines (YN75, YN-tobacco and Dzh-Qyg) and Italian isolate (74PAV) isolates(Fig. 2a) While, the amino acid sequence of CP gene of AARTPK clustered in a separate clad with chines isolates (Fig. 2b).

Recombination analysis

In recombination analysis by RDP 4, Pakistani isolate AARTPK was detected as recombinant between Pakistani (KT876049) and Indian (JN624776) isolates as major and minor parents respectively. The recombinant breakpoint was identified as, starting from 424th nucleotide and ending at 828th nucleotide (Fig. 3).

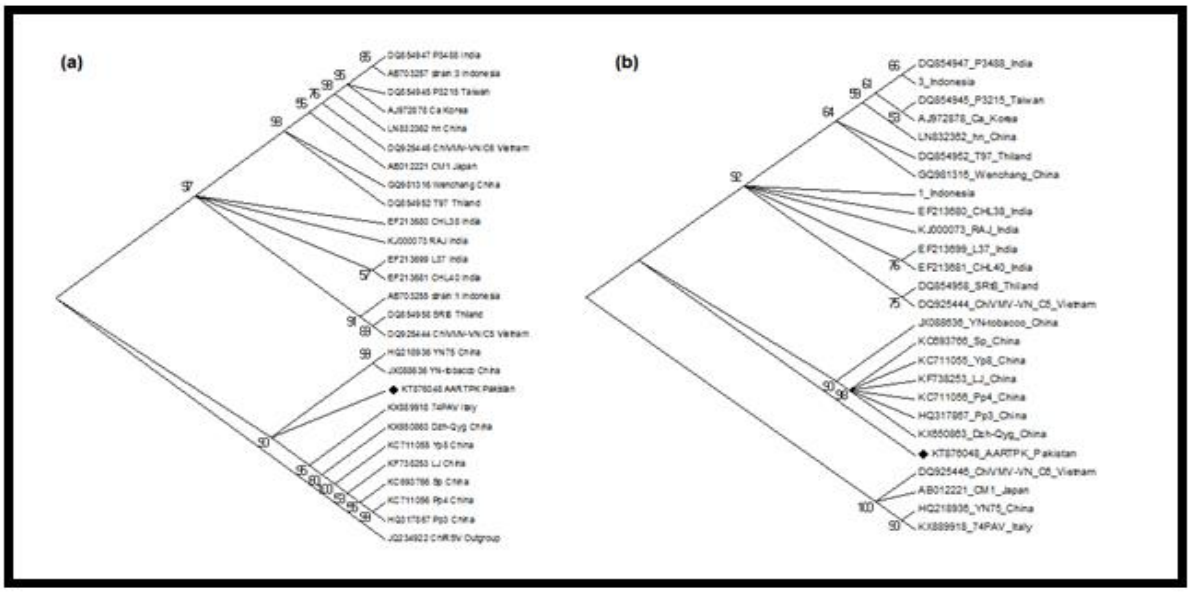

Fig. 2. Neighbour-joining phylogenetic trees constructed from nucleotides (a) and deduced amino acid (b) sequences of complete CP genes of26 ChiVMV isolates including AARTPK (dotted). Bootstrap analysis was done with 1000 replicates of the starting tree.

The recombination event was detected by five methods viz; RDP, BootScan, MaxiChi, Chemeara and 3Seq with average P-values of 1.239 x 10-03, 4.746 x 10-03, 3.276 x 10-05, 2.853 x 10-04 and 8.029 x 10-06 respectively.

Discussion

In present study, the ChiVMV isolate AARTPK was characterized based on nucleotide and amino acid sequence of CP gene and 3'UTR. Many pot viruses have been characterized on the bases of CP gene and 3' UTR (Fan *et al.,* 2003; Tsai *et al.,* 2008; Ha *et al.,* 2008). In taxonomy of pot viruses, amino acid and nucleotide sequences of CP gene and 3'UTR are considered as classification criteria and it states that the viruses with >80% CP gene amino acid identity are same species (Fauquet *et al.,* 2005).

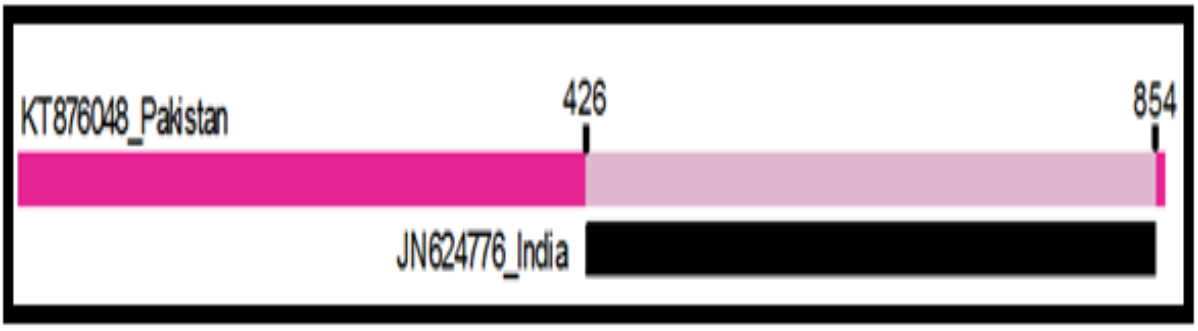

Fig. 3. Recombination event discovered by RDP 4 program in CP gene of AARTPK isolate. Substituted region from another virus isolate is represented by light pink bar, identity thereof given by a black bar.

Pakistani isolate AARTPK shared 88-96% nucleotide and % amino acid identity with other ChiVMV isolates, which verifies it as a strain of ChiVMV. Formerly, Frenkel *et al.* (1989) has also used the sequence of 3'UTR in classification of Potyvirus. Likewise, other potyviruses, the presence of poly adenylated tailat 3'end of genomic RNA of ChiVMV (Fauquet *et al.,* 2005), an inverted repeat sequence (GUGGNNNCCACC) in 3' end (Chiemsombat *et al.,* 1998), conserved region MVWCIENGTSP (Ward *et al.,* 1992) and DAG motif in the deduced amino acid sequence of CP gene of isolate AARTPK were also observed.

Such findings have also been described by Dombrovsky *et al.* (2005) and Ding *et al.* (2011). It verifies the AARTPK isolate as an aphid-transmitted ChiVMV potyvirus. The Pakistani isolate had a cleavage site Q/A, (between Nib and CP gene) which is which is supported by the findings of Tsai *et al.* (2008). The highest nucleotide identities, phylogenetic relationship and results of recombination analysis, strongly support the geographical affiliation of ChiVMV isolate AARTPK with India and China. The detection of recombinant event in isolate AARTPK is not a new case,a number of potyviruses has been investigated for possible recombination (Revers *et al.,* 1996). Above mentioned discussion suggests further studies on complete genome sequencing of ChiVMV for better understanding of its genetic variability.

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