Comparative coat protein annotation of two biologically distinct strains of Cucumber mosaic virus in chilli

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Research Article

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Abstract

Cucumber mosaic virus (CMV) has worldwide distribution with the broadest host range. Coat protein (CP) is the fundamental determinant for identification, symptom induction, systemic movement, encapsidation and aphid transmission of CMV. Molecular indexing explicated the prevalence of both mosaic inducing and chlorosis inducing strains of CMV in chilli growing tracts of Tamil Nadu. Precisely, CP consist of 657 nucleotides encodes for 218 amino acids was cloned and sequenced. The comparative amino acid analysis depicted Ser^{129} and Thr^{137} to be conserved in chlorosis inducing strains whereas Pro^{129} and Ser^{137} are conserved in mosaic inducing TN CMV strains. Moreover, chlorosis inducing strains was observed to have cryptic mutation of Thr^{162}, conserved for aphid transmission. Further, structural annotation of CP vindicated the propound modifications in secondary structure of protein. Moreover, TN CMV strains detected to implicit five functional domains in CP. Intriguingly, chlorosis inducing strains discerned to have disordered protein at functional motif of protein kinase K phosphorylation site. Remarkably, Casein kinase II phosphorylation site also observed in TN isolates which is purely conserved for CPs of all *Cucumovirus*. Besides, sequence diversity analysis postulate chlorosis inducing strains to have greater synonymous substitution over mosaic inducing strains and further phylogenetic analysis vindicated TN CMV isolates (mosaic and chlorosis inducing strains) belong to subgroup IB. The present study was taken to delve the genetic diversity of biologically distinct mosaic and chlorosis inducing strains of CMV infecting chilli in Tamil Nadu.

Introduction

Cucumber mosaic virus (CMV) is one of the destructive plant viruses with wider host range which infect utmost all vegetables crops (Kunkalikar et al. 2010). CMV was first reported back in 1916, ever since the notorious virus reported worldwide. About 36 viruses were reported to be infecting chilli and causing significant yield loss (Makkouk and Gumof 1974; Bhadramurthy et al. 2009). CMV (genus: *Cucumovirus*; family: *Bromoviridae*) harbor positive sense, single stranded and multi component virus with tripartite genomic RNA (Asad et al. 2019). RNA1 (~ 3.3 kb) encodes a replicase protein (1a) possess methyl transferase domain at N terminal and helicase motif at C terminal end. RNA2 (~ 3.0 kb) encodes larger replicase protein (2a) possess GDD motif for RNA dependent RNA polymerase (RdRp) and small silencing suppressor gene (2b) expressed from overlapping ORF2a. RNA 3 (~ 2.2 kb) encodes movement protein (3a) and coat protein (3b) expressed from sub genomic RNA (Jacquemond 2012). CMV is an ico-sahedral particle with 29nm in diameter composed of 180 identical sub units of CP (A, B and C) with quasi- three fold symmetry. Remarkably, sub unit A form pentameric capsomers and subunit B and C forms hexameric capsomers and has a β-barrel topology (Salanki et al. 2018).

CMV induces the broad spectrum of symptoms depending on virus strain and host such as mild to severe mosaic, yellowing, stunting, chlorosis, necrosis, leaf filiformity, malformation and fruit distortion (Montasser et al. 2017; Vinodhini et al. 2020). Broadly, CMV categorized into two major subgroups as subgroup I and subgroup II on the basis of peptide mapping of coat protein, nucleotide sequence homology and serological relationship. The subgroup I is further divided into subgroup IA and IB based

on the nucleotide variation at 5’ non coding region of coat protein (Dubey et al. 2010; Palukaitis et al. 1992). Furthermore, CMV strains categorized into chlorosis inducing strain and mosaic inducing strain based on amino acid position (A129) in CP (Shintaku 1991). It can be transmitted mechanically by sap and aphids (Montasser et al. 2011). CP of CMV considered as fundamental determinant for its role of encapsidation, symptom induction, host range, systemic movement and aphid transmission of the virus (Srivastava and Raj 2008). Among different plant viruses, CMV possess high degree of diversity with higher number of isolates which utters variability in molecular and biological properties of the virus (Jacquemond 2012). Besides, CMV prone to have high potential for population diversity like other single-stranded RNA virus (Ouedraogo et al. 2019). Synonymous and non-synonymous amino acid mutation in viral genes may alter the structure and functions of proteins and also implicate directly in virus and host plant interaction (Mochizuki et al. 2018). A deleterious change in secondary structure of coat protein results in reduction of viral RNA titer and stability of virus. Moreover, amino acid mutations may gravely alter the structure and functions of viral protein (Mochizuki et al. 2018). CMV undergoes ample of genetic recombination, mutation and reassortment comparatively than other plant viruses. Thus, rapid evolution of CMV prompts to emergence of new strains and better adopted gene to extend their host range, stability and infectivity (Ouedraogo et al. 2019; Vinodhini et al. 2021). Hence, understanding the complex of genetic structure and diversity is crucial for the development of better viral disease management (Nouri et al. 2014; Pavithra et al. 2019; Vinodhini et al. 2020). The main objective of this study is to comprehend the coat protein diversity and the structural annotation of mosaic inducing and chlorosis inducing strains of CMV infecting chilli.

Materials And Methods

Virus isolates

A diagnostic survey was conducted during 2019 to assess the prevalence of CMV in major chilli growing areas of Tamil Nadu. The virus infected chilli plants showing mosaic, mosaic mottling, leaf filiformity (shoe string), chlorosis, necrosis and leaf malformation with stunted growth were collected. The virus inocula of CMV isolates maintained on *Nicotiana benthamiana* and *Luffa acutangula* by sap inoculation under insect proof greenhouse of Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore (India) for further analysis. The preliminary confirmation of CMV in the sample was done through RT-PCR analysis and DAC-ELISA.

Reverse Transcription-polymerase Chain Reaction (Rt-pcr)

The total RNA extracted from CMV inoculated host plants using TRIzol reagent (Chomczynski and Sacchi 2006). The first strand cDNA was synthesized by preparing transcription mixture (9µl of sterile water, 4µl of 5× reaction buffer, 2µl of dNTPs, 1µl of random primer, 1µl of reverse transcriptase and 1µl of RNase inhibitor and 1µg of total RNA) with condition of 42ºC for 60 min followed by 70ºC for 5 min incubation (Chomczynski and Sacchi 2006; Asad et al. 2019). The presence of CMV was confirmed through RT-PCR

amplification using CP gene specific primers (GK CMVCP F 5'GAGTTCTTCCGCGTCCCGCT3'; GK CMV CP R 5' AAACCTAGGAGAT GGTTTCA 3') with cyclic parameters of 94ºC for 2 min followed by 94ºC for 30 sec of denaturation, 54ºC for 30 sec of annealing and 72ºC for 90 sec followed by final extension of 72ºC for 10 min (Ramesh and Sreenivasalu 2018; Nagendran et al. 2018).

Cloning And Sequencing

The amplified CP was excised from the gel and purified with GenJET PCR purification kit (Themo scientific Inc.) as per manufacturer's protocol. The purified coat protein gene was ligated into pGEM-T easy vector (Promega) and transformed into *Escherichia coli* DH5α strain. The consequent recombinant clones were selected on Luria Bertoni medium containing 100mg/l of ampicillin, X-gal and IPTG (Sambrook and Russell 2001). Subsequently, consensus fragment sequenced at both orientation at M/s Barcode Bioscience, Bangalore.

Sequence Analysis

The concise coat protein gene of 657 nucleotides were determined using Gene runner 6.0 (www.generunner.net) and sequences of CMV subgroup IA, IB and II isolates reported from different parts of world have been retrieved from NCBI database (http://www.ncbi.nlm.nih.gov/BLAST) (Table S1). Nucleotide sequences translated into amino acid residues by Expasy tool and multiple alignments carried out with Clustal W (www.ebi.ac.uk) and MultAlin (sacs.ucsf.edu) to detect variability among the amino acids of coat protein. Further, sequence alignment editing and consensus sequence logo designing was carried out using Jalview 2.11.1.4. Indeed, pairwise sequence analysis was carried out using Sequence Demarcation (SDTv 1.2) software (http://web.cbio.uct.ac.za) and nucleotide diversity analysis performed using DnaSP ver. 5.10. Nucleotide diversity among TN isolates were analyzed from estimating genetic distance by Kimura's two-parameters method and compared using non parametric Wilcoxon test with 1000 replication of bootstrap method (Bergua et al. 2014). The per cent identity matrix was calculated using Genomatix (http://www.genomatix.de/) and phylogenetic tree was constructed by Neighbor joining tree method with 1000 bootstrap replicates using MEGA 7.0 software (www.megasoftware.net) (Bhadramurthy et al. 2009; Kumar et al. 2016).

Structural Annotation

The sequence based homology search in RCSB protein data bank (http://www.rcsb.org/pdb) carried out to find the protein structure with relatively close homologous to chlorosis inducing (BTN1) and mosaic inducing strains (BTN5) of CMV. Based on the homology modeling, secondary structure of coat protein was designed with SWISS MODEL program (http://swissmodel.expasy.org) using Fny-CMV strain (PDB ID: 1F15) as template structure with the target protein sequences. Further, designed coat protein structure validated for stereochemistry using Ramachandaran plot. Based on the percentage of favored and

generously allowed amino acids, frequency of outliers and dihedral angle of φ against ψ, the model protein structures were selected and further used for analysis (Lovell et al. 2003). Moreover, super imposed protein model of BTN1 and BTN5 strains were designed using Superpose 2.1 program (http://superpose.wishartlab.com) to explore the distinct quaint variation between secondary coat protein structure of BTN1 and BTN5 strains. Further annotation of secondary protein structure prediction performed with PSIPRED, disorder protein prediction using DISOPRED3 in the intervening position of sequence, protein domain prediction with PROSITE and Membrane Helix Prediction using MEMSAT-SVM (http://bioinf.cs.ucl.ac.uk).

Results

The notorious virus recorded to prompt 50-60% of disease incidence over Tamil Nadu in chilli. The characteristic symptoms of mild to severe mosaic, mosaic mottling, leaf filiformity (shoe string), chlorosis and stunted growth were observed (Fig. 1). The presence of CMV in the infected plant samples were confirmed through RT-PCR using CP gene specific primer. The products of 1200-bp were amplified, cloned and then sequenced (Figure S1). Therefore, five CMV isolates (MT396263; MT396265; MT395346; MZ093618; MZ093617) showed greater variation has been used in the study. Nucleotide sequences of CP (657nt) was compared and evaluated to comprehend genetic properties and variability in amino acids. Comparatively, TN isolates MT396263 (BTN1), MT396265 (BTN2) and MT395346 (BTN3) shared 98.28- 98.48% identity with MZ093618 (BTN4) and MZ093617 (BTN5). The nucleotide sequences of TN CMV isolates shared 97.65-98.92% homology with Indian chilli isolates (HM348786; KM272275; KJ645896; MT396265; KM272276; KJ645897; KX9611535). The heat map generated using SDTv1.2 explicated that TN isolates shared amino acid homology of 97-99% identity with subgroup IB, 93-95% identity with subgroup IA and 75-79% identity with subgroup II of CP respectively (Fig. 2). Moreover, TN isolate of BTN1 shared 99.5% identity with BTN2 and BTN3 isolates. Similarly, 96.7% identity with BTN4 and 97.2% identity with BTN5 of TN isolates. The pairwise percent identity of TN isolates with other members of CMV isolates were presented in Table 1.

Deduced amino acid sequence analysis of complete coat protein

Any variation in a single amino acid may catastrophically affect the virulence, transmission, symptomatology and stability of virus. The multiple alignments of deduced CP amino acid sequences explicate substitution, deletion and conserved regions among different strains of CMV. There are five subgroup IA strains, seven subgroup IB strains and five subgroup II representative strains were used for comparative sequence analysis (Fig. 3). Comparative amino acid sequence analysis depicted Phe^{99} amino acid to be conserved in subgroup IB strains, whereas subgroup IA and II strains possess Tyr^{99} amino acid respectively. The significant role of the amino acid was not well understood. Indeed, 30 amino acids were found to be conserved among subgroup I strains comparatively with subgroup II strains. Similarly, amino acids (Lys^{65} and Lys^{82}) at the position of 65 and 82 observed to be conserved among subgroup IB and subgroup II strains. Besides, conserved basic ARM (Arginine Rich Motif) observed at 5 prime end (14-24 position) of CP in subgroup I and II strains. Therefore, deletion of amino

acid threonine (Thr^{12}) observed at 12^{th} position of TN isolates (subgroup IB) subsequently in subgroup I strains as well. It suggests that Thr^{12} amino acid conserved for CMV subgroup I strains alone. Similarly, deletion was observed in 25^{th} position of subgroup II strains. In contemplate, subgroup IA strains possess proline (Pro^{25}) and subgroup IB strains possess serine (Ser^{25}) at 25^{th} position.

Notably, unique amino acid substitution observed at 129^{th} position of CP (Pro^{129} and Ser^{129}). Whereas some of isolates possess Pro^{129} (BTN1; BTN2; BTN3) and some of possess Ser^{129} (BTN4; BTN5). It depicts that CMV isolates possess Pro^{129} are mosaic inducing strains (BTN1; BTN2; BTN3) and thus substituted with Ser^{129} are chlorosis inducing stains (BTN4; BTN5). Hence it evinces the existence of chlorosis and mosaic inducing strains of CMV in chilli growing areas of Tamil Nadu. Indeed, mosaic and chlorosis symptom induction bestowed by conserved amino acid 129 (Ser^{129} and Pro^{129}). Moreover, quaint amino acid substitution (Thr^{137}) observed over 137^{th} position in chlorosis inducing TN strains comparatively with other mosaic inducing strains of subgroup IB members (Ser^{137}). Hence, the result implies that amino acids Ser^{129} and Thr^{137} to be conserved in CP of chlorosis inducing TN strains. In contemplate, amino acids of Pro^{129} and Ser^{137} to be conserved in mosaic inducing strains of CMV. Similarly, aphid vector transmission conferred by conserved alanine (Ala^{162}) at the position of 162. Empirically, some of chlorosis inducing strains also showed amino acid substitution of Thr^{162} over conserved Ala^{162}.

Structural annotation of CP

The amino acid sequence based structural annotation has been carried out for CP of mosaic inducing (BTN1) and chlorosis inducing strain (BTN5) using Swiss model and validated by Ramachandran plot. The results postulate that CP has canonical topology comprised of 180 sub units dispersed as 60 copies in three (A, B and C) subunits with threefold symmetry. Moreover, subunit A possess pentameric capsomer whereas subunit B and C form hexameric capsomer. Speculatively, those three subunits observed to have identical amino acid sequences with little different conformations. A Notable modification in secondary structure of CP was detected in BTN5 (chlorosis inducing stain) when compared with BTN1 (mosaic inducing strain). Presumably, βB- βC loop positioned at 76-83 residues, βD-βE loop at 113-119 residues, βE- αEF loop at 129-136 residues, βF-βG loop at 155-163 residues and βH-HI loop at 191-199 resides in coat protein of CMV.Intriguingly, the super imposed structure of BTN1 and BTN5 vindicated the structural loop modification of βE-αEF (129 residue), βF-βG (162 residue) and βB-βC loops (78 residues) in coat protein of CMV (Fig. 4). The corresponding identical and non-similar amino acids in the superimposed protein structure based on dihedral angle were depicted in Ramachandaran plot (Fig. 5).

Furthermore, confound positional modification of helix domain was observed at 170-172 amino acid of BTN5 whereas BTN1 confer at 171-172 amino acids. Similarly, confound modification of β sheets observed at 106- 108 amino acid of BTN5 whereas BTN1 confer at 106-110 amino acids. Amicably, BTN5 flaunted disordered protein binding domain while compared with BTN1. The graphical representation of

helix, β sheets, coil and disordered protein binding laid on amino acid position of BTN1 and BTN5 of CP have been depicted graphically (Fig. 6). Furthermore, amino acid sequence based analysis of domain identification algorithm explicated the presence of functional motif of glycosylation (NTSS) and phosphorylation sites (TcR, StK, TIK, SIK, StcE, SsiD and SviE) in CP (Table 2). Distinctly, upon PROSITE functional site search, Casein kinase II phosphorylation site also observed in CP which is purely conserved for CPs of all *Cucumovirus*.

Sequence diversity

Furthermore, diversity analysis of CP using DnaSP ver.5.10 explicates haplotype diversity of TN isolates as one and number of segregation sites observed as 449. Comparatively, greater synonymous and non-synonymous substitution of nucleotides observed in TN chlorosis inducing strains than mosaic inducing strains. Moreover, cumulatively significant synonymous substitutions were discerned in CP of TN isolates over non-synonymous substitution. Over all, it implies that chlorosis inducing strains having higher nucleotide diversity than of mosaic inducing strains of CMV (Table 3).

Phylogenetic analysis

Phylogenetic relationship of CP of TN isolates evaluated with subgroup I and II members of CMV isolates reported from different parts of world. The phylogenetic tree generated with deduced CP amino acid sequences of CMV isolates using Mega 7 program with 1000 replicates of bootstrap values. Besides, Fny, M, Y and Tfn strains of CMV were taken as standard subgroup I reference strains and Trk7II strain taken as subgroup II reference strain. Alike, Peanut stunt virus (U15730) included as out group for rooting in the phylogeny. Phylogenetic analysis explicates the formation of two different cluster forsubgroup I and subgroup II (Fig. 7). Moreover, TN isolates shared high vicinity with members of subgroup I. Precisely, TN isolates clustered together with subgroup IB strains, well separated from subgroup IA. While, subgroup IA strains were clustered with Fny, M, Y and Tfn reference strains and subgroup II clustered with Trk7II reference strain of CMV. It clearly depicts that mosaic inducing and chlorosis inducing strains of TN CMV isolates were belonging to subgroup IB.

Discussion

Molecular characterization aids in giving an insight about genetic properties, composition and variations inflicted by mutations and recombination. The presence of CMV in infected plants was confirmed through RT-PCR analysis. Since, RT-PCR based detection is rapid and sensitive to detect plant virus rather than serological based assay (Hu et al. 1995; Dubey et at. 2010). CP gene of CMV was amplified using gene specific primers and determined to comprise of 657nt encoded into 218aa (Nagendran et al. 2018; Dubey et at. 2010; Pavithra et al. 2019; Vinodhini et al. 2020). CP regarded as multifunctional protein for its entail in symptom development, encapsidation, host range, vector transmission, virion stability and systemic movement of CMV (Takahashi et al. 2001; Mochizuki and Ohki 2012). Sequence comparison suggests that subgroup I and subgroup II differ by 30 amino acids. Similarly, subgroup IA and subgroup IB differ by 5–7 amino acids. Comparative sequence analysis helps to undermine crucial role of amino

acid interplay over transmission, symptom induction and stability of virus (Taliansky et al. 1995; Shintaku et al. 1992; Suzuki et al. 1995).

Indeed, amino acid arginine (Arg) at 5 prime ends (14–24 position) to be conserved in subgroup I and II CMV strains. N terminus of CP is basic arm contains highly conserved arginine rich motif (ARM) which maintain the structural integrity and stability of virus (Osman et al. 1998). The amino terminus plays a key role in conserving structural integrity of CP in aid of nurturing compatible host plant interaction (Rao 1997). On the other hand, clusters of basic amino acids in N terminal region implicated in coat protein and RNA interactions of virion (Harrison 1984 and Perry et al. 1998). It suggests that ARM region to be highly conserved among CMV strains. Dubey et al. (2010) also stated that amino acid arginine (Arg^{16}) at 16th position to be highly conserved among all of CMV strains and play a vital role in viral RNA and CP interactions. The mutation deletion observed in 25th position in subgroup II and 12th position in subgroup I CMV strains of CP. In addition, comparative amino acid sequence analysis explicates that Phe^{99}, Ser^{137} and Gly^{144} amino acids to be conserved among subgroup IB strains. Similarly, Dubey et al. (2010) expound that tyrosine (Tyr) at 99th position to be conserved over subgroup IA and II strains of CMV, whereas Indian isolates (subgroup IB) substituted by phenylalanine (Phe).

Furthermore, deduced amino acid sequence analysis vindicated the distinct amino acid substitution at 129th position of CP (Pro^{129} and Ser^{129}). Shintaku et al. (1991) affirms that residue of 129th amino acid of CP is a determinant of symptom induction and movement of virus. More precisely, amino acid proline (Pro^{129}) induces mosaic symptom and thus substituted with serine or leucine (Ser^{129} or Leu^{129}) induces chlorosis symptom in the infected plants. Accumulated reports propound amino acid 129 to be situated at foremost position of βE-αEF loop (129–136 residues) in CP. Indeed, flexibility of thus loop depends solely on the properties of residue 129 whereas increased flexibility associated with pathogenesis of virus (Bhardwaj and Purohit 2020; Gellert et al. 2006; Mochizuki and Okhi 2012; Vinodhini et al. 2021). The structural alteration inflicted by amino acid substitution (Ser^{129}) induces chlorosis in the infected plant by impairing chloroplast by suppressing the accumulation of ferredoxin I (Suzuki et al. 1995; Qiu et al. 2018). Correspondingly, amino acid 129 (Pro^{129}) of CP is a determinant for successful SAM (shoot apical meristem) invasion which implicit in cell to cell movement of virions (Mochizuki and Okhi 2005; Valentine et al. 2002; Wong et al. 1999). Furthermore, quaint amino acid substitution of Thr^{137} observed in chlorosis inducing strains of TN isolates comparatively with other mosaic inducing strains of subgroup IB members (Ser^{137}). Similarly, apparent amino acid substitution (Ser^{129} and Thr^{137}) reported in chlorosis inducing strain of CMV causing mixed infection with tospovirus (Vinodhini et al. 2021). The pervasive perceptions suggest that amino acid residue of Ser^{129} and Thr^{137} to be conserved in chlorosis inducing strains of TN isolates. In contrast, amino acids Pro^{129} and Ser^{137} are conserved in mosaic inducing strains of TN CMV isolates.

Efficiency of transmission solely depends on composite factors of virus strain, vector aphid and accumulation of virion particles in host. Variation in transmissibility of different CMV isolates is driven by CP amino acid determinant (Arenal and Palukaitis 2009). Aphid transmission of CMV solely determined

by the distinct conserved amino acid alanine (Ala^{162}) and the mutation in a single amino acid significantly affect the transmission of virus (Dubey at al. 2010). Indeed, most of mosaic inducing strains of CMV possess Ala^{162} and in contrast, some of chlorosis inducing strains own cryptic mutation of Thr^{162}. Interestingly, isolates (BTN4 and BTN5) having potent mutation of Ser^{129} also postulate amino acid substitution of Thr^{162} in CP. Amino acid 162 located on βF–βG loop, hence variability inflicted on the potent amino acid disrupt the salt bridges (Lys^{101}-Asp^{178}) between sub units which destabilizes the quasi three fold symmetry of CP (Ng et al. 2000 and Bricault and Perry 2013). Thus, implies in reduction of virus stability which may have effect on vector transmission of CMV. Probably, disruption of surface charge or rather structure of CP inflicted by reassortant could lead to loss of nature of aphid borne transmission of virus (Pierrugues et al. 2007; Jacquemond 2012). Some experimental reports also shown that the mutant strains of CMV by modifying alanine (Ala^{162}) as threonine (Tyr^{162}) in CP results in loss of transmissibility and stability of virus (Ng et al. 2000). Amicably, single structural modification imply on antigenicity of virus may amend the ability of virus to bind with vector. On other hand, population bottleneck congestion during vector transmission may also evoke the genetic drift such ultimately prompt to differential strains in virus (Ali et al 2006).

Further, structural annotation explicated confound positional modification of helix domain at 170–172 residues of chlorosis inducing strain and while mosaic inducing strain confer at 171–172 amino acids. Similarly, β sheets observed to be laid at 106–108 residues of chlorosis inducing strain whereas mosaic inducing strains confer at 106–110 amino acids. As intuitively, chlorosis inducing strains exhibited disordered protein binding domain. The disordered proteins are instrumental in cell signaling, protein-protein interaction and binding (Japrung et al. 2013). There are four functional motif observed to be present in CP of all TN CMV strains which helps to sustain the CMV infection in host plant. The disordered proteins observed to be present at functional motif of protein kinase K phosphorylation site (8–10 amino acids). Although, distinct role of the association of disordered proteins is yet to be studied. Empirically, casein kinase II phosphorylation site also observed in CP of TN strains which is strictly conserved for CPs of all *Cucumovirus* members (Salanki et al. 2018). Indeed, potential phosphorylation of protein is the basis to establish infection by CMV strains (Lauring et al. 2012). Nucleotide diversity analysis revealed that TN isolates to be having greater synonymous substitution over non-synonymous substitutions. Synonymous mutations in viral genes will results in alteration of secondary structure of RNA and codon usage bias (Mochizuki et al. 2018). Moreover, synonymous substitutions plays crucial role in virulence, adaptation and evolution of virus (Kim and Palukaitis 1997; Mochizuki et al. 2018). Distinctly, chlorosis inducing strains detected to have higher synonymous substitution over mosaic inducing strains.

The concurrent study evinces the dynamic role of multifunctional CP in symptom (mosaic or chlorosis) induction and stability of virus. Moreover, occurrence of mosaic inducing and chlorosis inducing strains of CMV has been documented in chilli growing tracts of Tamil Nadu. Interestingly, chlorosis inducing strains of CMV with potent amino acid variation observed to be causing mixed infection with tospoviruses rather mosaic inducing strains causes single infection. Empirically, CMV go through swift of

genetic drift through recombination and reassortment which lead to the genetic changes or variability and purge the deleterious mutations. Therefore, reassortment of genetic segment between related viruses profusely inflict to the emergence of some newer strain or variant. Hence, accumulating evidences postulates the genetic diversity observed on mosaic inducing strain implies the selection pressure imposed on CMV during co-adoption with other virus might be eventually prompt to the occurrence of chlorosis inducing strain. The present study sought to expound the genetic diversity and coat protein structure of mosaic and chlorosis inducing strains of CMV infecting chilli in Tamil Nadu. Moreover, understanding the nexus of diversity and population structure of CMV is pivotal in developing better disease management strategies.

Declarations

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Author contribution statement

GK planned, designed and supervised the study, revised the manuscript and approved final manuscript for submission; JV conducted the experiments, analyzed the data and prepared the manuscript; DJSS helped for the computational analysis and LR gave proposition to conduct the experiment. All the authors reviewed and approved the submission.

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References

1. Ali A, Li H, Schneider WL, Sherman DJ, Gray S, Smith D, Roossinck MJ (2006) Analysis of genetic bottlenecks during horizontal transmission of Cucumber mosaic virus. Journal of Virology 80(17): 1–6.

2. Asad Z, Ashfaq M, Ahsan M (2019) Serological and Molecular Identification Based on Coat Protein (CP) Gene of Cucumber mosaic virus (CMV) Infecting Cucumber (*Cucumis sativus* L) in Pothwar Region of Pakistan. Journal Plant Biochemistry and Physiology 7: 1–5
3. Bergua M, Luis-Arteaga M, Escriu F (2014) Genetic diversity, reassortment, and recombination in Alfalfa mosaic virus population in Spain. Phytopathology 104 (11): 1241–1250.
4. Bhadramurthy V, Bhat AI (2009) Biological and molecular characterization of Bean common mosaic virus associated with vanilla in India. Indian Journal of Virology 20 (2): 70–77.
5. Bhadramurthy V, George A, Bhat AI, Shiva KN (2009) Coat protein gene sequence studies suggest that Cucumber mosaic virus infecting paprika (*Capsicum annuum* L.) in India belongs to subgroup IB. Archives of Phytopathology and Plant Protection 42: 857–863.
6. Bhardwaj VK, Purohit R (2020) Structural changes induced by substitution of amino acid 129 in the coat protein of Cucumber mosaic virus. Genomics 112: 3729–3738.
7. Bricault CA, Perry KL (2013) Alteration of intersubunit acid–base pair interactions at the quasi-threefold axis of symmetry of Cucumber mosaic virus disrupts aphid vector transmission. Virology 440: 160–170.
8. Chomczynski P, Sacchi N (2006) The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. Nature protocols 1: 581–585.
9. Dubey VK, Singh VP (2010) Molecular characterization of Cucumber mosaic virus infecting *Gladiolus*, revealing its phylogeny distinct from the Indian isolate and alike the Fny strain of CMV. Virus Genes 41: 126–134.
10. Garcıa-Arenal F, Palukaitis P (2009) Cucumber mosaic virus. Desk Encyclopedia of Plant and Fungal Virology 171: 1–6.
11. Gellert A, Salanki K, Naray-Szabo G, Balazs E (2006) Homology modelling and protein structure based functional analysis of five *Cucumovirus* coat proteins. Journal of Molecular Graphics and Modelling 24: 319–327.
12. Harrison SC (1984) Multiple methods of subunit association in the structure of simple spherical viruses. Trends in Biochemical Sciences 9: 345.-351.
13. Hu JS, Li HP, Barry K, Wang M, Jordan R (1995) Comparación de las pruebas" dot blot", ELISA y RT-PCR para la detección en dos aislados del virus del mosaico del pepino que infecta los bananos en Hawai. Plant Disease 79: 902–906
14. Jacquemond, Mireille (2012) Cucumber mosaic virus. Advances in Virus Research 84: 439–504.
15. Japrung D, Dogan J, Freedman KJ, Nadzeyka A, Bauerdick S, Albrecht T, Kim MJ, Jemth P, Edel JB (2013) Single-molecule studies of intrinsically disordered proteins using solid-state Nano pores. Analytical Chemistry 85: 2449–2456.
16. Kim CH, Palukaitis P (1997) The plant defense response to Cucumber mosaic virus in cowpea is elicited by the viral polymerase gene and affects virus accumulation in single cells. European Molecular Biology Organization Journal 16: 4060–4068.

17. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874.
18. Kunkalikar SR, Sudarsana P, Rajagopalan P, Zehr UB, Ravi KS (2010) Biological and molecular characterization of Capsicum chlorosis virus infecting chilli and tomato in India. Archives of Virology 155: 1047–1057.
19. Lauring, AS, Acevedo A, Cooper SB, Andino R (2012) Codon usage determines the mutational robustness, evolutionary capacity, and virulence of an RNA virus. Cell host & Microbe 12(5): 623–632.
20. Lovell SC, Davis IW, Arendall WB, De Bakker PI, Word JM, Prisant MG, Richardson JS, Richardson DC (2003) Structure validation by Cα geometry: ϕ, ψ and Cβ deviation. Proteins: Structure, Function, and Bioinformatics 50: 437–450.
21. Makkouk KM, Gumof DJ (1974) Virus diseases of pepper in California. Plant Disease Report 58: 1002–1006.
22. Mochizuki T, Ohara R, Roossinck MJ (2018) Large-Scale synonymous substitutions in Cucumber mosaic virus RNA 3 facilitate amino acid mutations in the coat protein. Journal of Virology 92: 1–13.
23. Mochizuki T, Ohki ST (2005) Amino acid 129 in the coat protein of Cucumber mosaic virus primarily determines invasion of the shoot apical meristem of tobacco plants. Journal of General Plant Pathology 71: 326–332.
24. Mochizuki T, Ohki ST (2012) Cucumber mosaic virus: viral genes as virulence determinants. Molecular Plant Pathology 13: 217–225.
25. Montasser MS, Dashti NH, Ali NY (2011) Attenuation of viral symptoms and yield reduction responses to CMV infection in tomato crops subjected to plant growth promoting rhizobacteria. Federation of American Societies for Experimental Biology Journal 25: 714–765
26. Montasser NN, Nayef NY, Afzal M (2017) Photosynthetic Apparatus and Biochemical Parameters in Tomato and Squash Crops Influenced by Cucumber Mosaic Virus (CMV) Infection. Federation of American Societies for Experimental Biology Journal 31: 623–1
27. Nagendran K, Priyanka R, Aravintharaj R, Balaji CG, Prashant S, Basavaraj B, Mohankumar S, Karthikeyan G (2018) Characterization of Cucumber mosaic virus infecting snake gourd and bottle gourd in India. Physiological and Molecular Plant Pathology 103: 102–106.
28. Ng JC, Liu S, Perry KL (2000) Cucumber mosaic virus mutants with altered physical properties and defective in aphid vector transmission. Virology 276: 395–403.
29. Nouri S, Arevalo R, Falk BW, Groves RL (2014) Genetic structure and molecular variability of Cucumber mosaic virus isolates in the United States. PLoS ONE 9: 1–12
30. Osman F, Choi YG, Grantham GL, Rao ALN (1998) Molecular studies on Bromovirus capsid protein: V. Evidence for the specificity of Brome mosaic virus encapsidation using RNA3 chimera of Brome mosaic and Cucumber mosaic viruses expressing heterologous coat proteins. Virology 251: 438–448.

31. Ouedraogo RS, Pita JS, Somda IP, Traore O, Roossinck MJ (2019) Impact of cultivated hosts on the recombination of Cucumber mosaic virus. Journal of Virology 93: 1–9.
32. Palukaitis P, Roossinck MJ, Dietzgen RG and Francki RI (1992) Cucumber mosaic virus. Advances in virus research 41: 281–348
33. Pavithra BS, Govin K, Renuka HM, Krishnareddy M, Jalali S, Samuel DK, Himabindu K (2019) Characterization of Cucumber mosaic virus infecting coleus (*Plectranthus barbatus*) in Karnataka. Virus Disease 30: 403–412
34. Perry KL, Zhang L, Palukaitis P (1998) Amino acid changes in the coat protein of Cucumber mosaic virus differentially affect transmission by the aphids *Myzus persicae* and *Aphis gossypii*. Virology 242: 204–210.
35. Pierrugues O, Guilbaud L, Fernandez-Delmond I, Fabre F, Tepfer M, Jacquemond M (2007) Biological properties and relative fitness of inter-subgroup Cucumber mosaic virus RNA 3 recombinants produced *in vitro*. Journal of General Virology 88: 2852–2861.
36. Qiu Y, Zhang Y, Wang C, Lei R, Wu Y, Li X, Zhu S (2018) Cucumber mosaic virus coat protein induces the development of chlorotic symptoms through interacting with the chloroplast ferredoxin I protein. Scientific Reports 8: 1–11.
37. Ramesh B, Sreenivasulu P (2018) Detection of cucumber mosaic virus in brinjal and chilli by RT-PCR. International Journal of Pharmacy and Biological Sciences 8: 128–131.
38. Rao ALN (1997) Molecular studies on bromovirus capsid protein. Virology 232: 385–395.
39. Salanki K, Gellert A, Nemes K, Diveki Z, Balazs E (2018) Molecular modeling for better understanding of *Cucumovirus* pathology. Advances in Virus Research 102: 59–88.
40. Sambrook J, Russell DW (2001) Molecular Cloning: A Laboratory Manual. 3rd Edition, Cold Spring Harbor Laboratory Press, New York. 1.
41. Shintaku M (1991) Coat protein gene sequences of two Cucumber mosaic virus strains reveal a single amino acid change correlating with chlorosis induction, Journal of General Virology 72: 2587–2589.
42. Shintaku MH, Zhang L, Palukaitis P (1992) A single amino acid substituition in the coat protein of Cucumber mosaic virus induces chlorosis in tobacco. Plant Cell 4: 751–757.
43. Srivastava A, Raj SK (2008) Coat protein-mediated resistance against an Indian isolate of the Cucumber mosaic virus subgroup IB in *Nicotiana benthamiana*, Journal of Biosciences. 33 (2008) 249–257.
44. Suzuki M, Kuwata S, Masuta C, Takanami Y (1995) Point mutations in the coat protein of Cucumber mosaic virus affect symptom expression and virion accumulation in tobacco. Journal of General Virology 76: 1791–1799
45. Takahashi H, Suzuki M, Natsuaki K, Shigyo T, Hino K, Teraoka T, Hosokawa D, Ehara Y (2001) Mapping the virus and host genes involved in the resistance response in Cucumber mosaic virus-infected *Arabidopsis thaliana*. Plant and Cell Physiology 42: 340–347.

46. Taliansky ME, Garcia-Arenal F (1995) Role of *Cucumovirus* capsid protein in long-distance movement within the infected plant. Journal of Virology 69: 916–922.
47. Valentine TA, Roberts IM, Oparka KJ (2002) Inhibition of Tobacco mosaic virus replication in lateral roots is dependent on an activated meristem-derived signal. Protoplasma 219: 184–196.
48. Vinodhini J, Rajendran L, Abirami R, Karthikeyan G (2021) Co-existence of chlorosis inducing strain of Cucumber mosaic virus with tospoviruses on hot pepper (*Capsicum annuum*) in India. Scientific Reports 11: 1–9.
49. Vinodhini J, Rajendran L, Raveendran M, Rajasree V, Karthikeyan G (2020) Characterization of Cucumber mosaic virus (CMV) subgroup IB infecting chilli in Tamil Nadu, India. 3 Biotech 10: 1–10.
50. Wong SM, Thio SSC, Shintaku MH, Palukaitis P (1999) The rate of cell-to-cell movement in squash of Cucumber mosaic virus is affected by sequences of the capsid protein. Molecular plant-microbe Interactions 12: 628–632.

Tables

Tables 1 to 3 are available in the Supplementary Files section

Figures

Fig.1

Figure 1

The distinct mosaic symptoms induced by chlorosis inducing (a&b) and mosaic inducing strains (c&d) of CMV on chilli

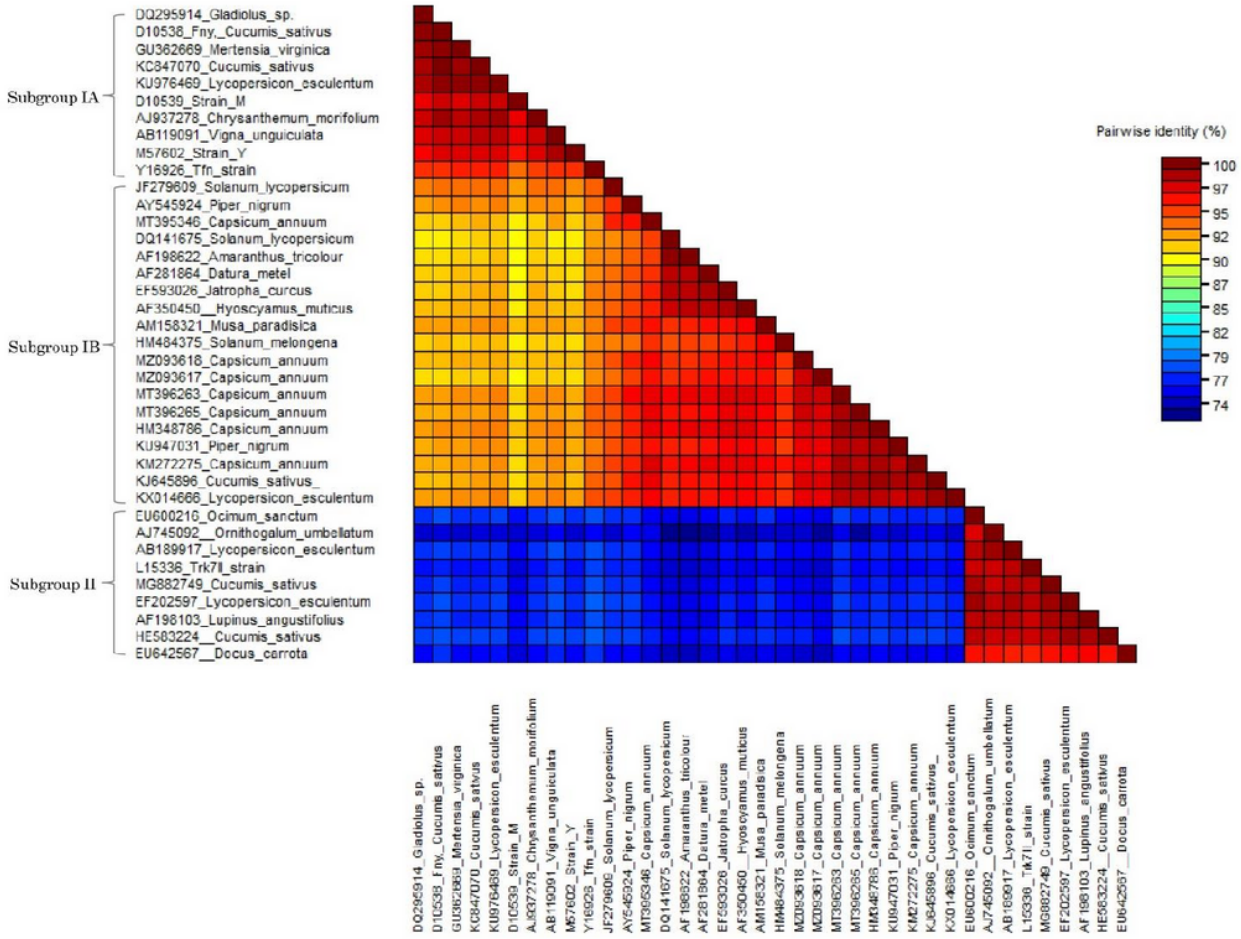

Fig. 2

Figure 2

Heat map representation of pairwise identity matrix of TN CMV isolates with other subgroup IA, IB and II members reported worldwide. The each colored cells illustrates the correspondence of pairwise identities between the different isolates. Moreover, the colored key represents per cent identity score between the isolates. The Amino acid based pairwise sequence identity scores were inferred using Sequence demarcation tool (SDTv 1.2)

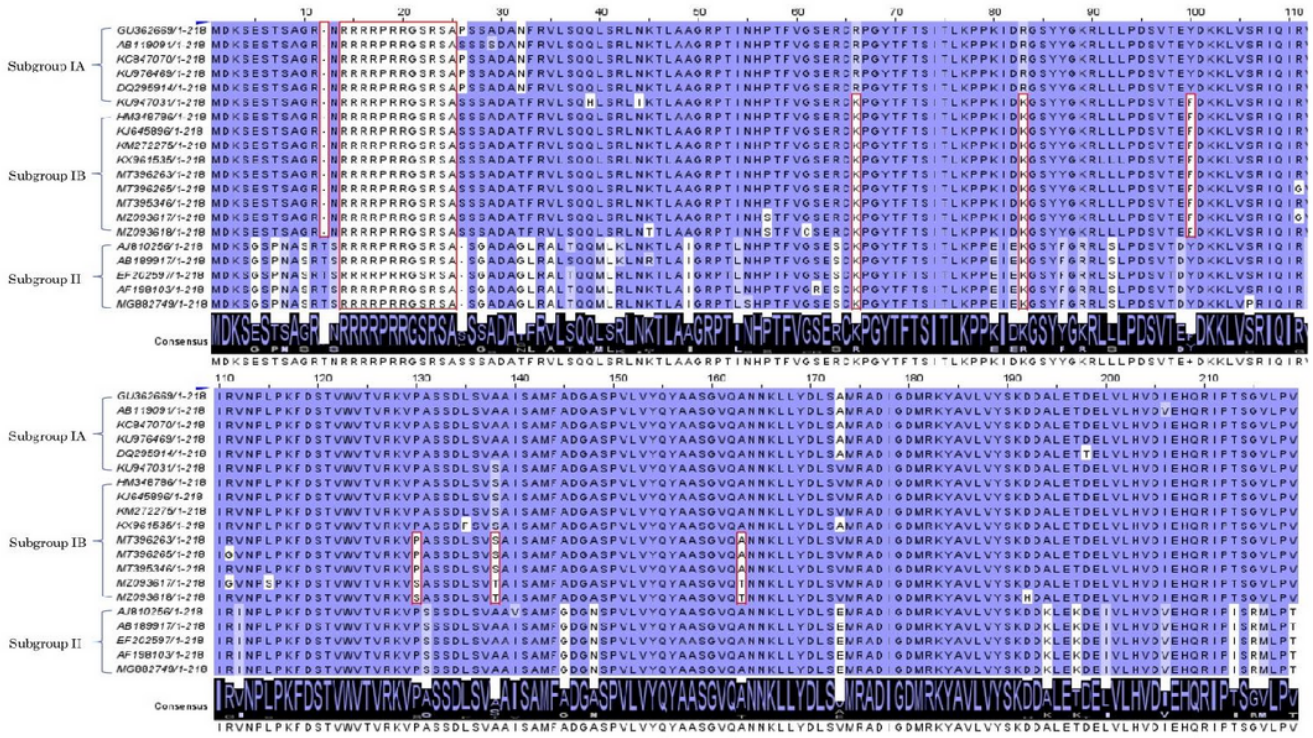

Fig. 3

Figure 3

Comparative multiple alignment analysis of complete coat protein gene (218aa) of TN CMV isolates with reference CMV strains belong to subgroup IA, subgroup IB and subgroup II at amino acid level. The simplified sequence logo of CP illustrates the consensus amino acid among the different strains

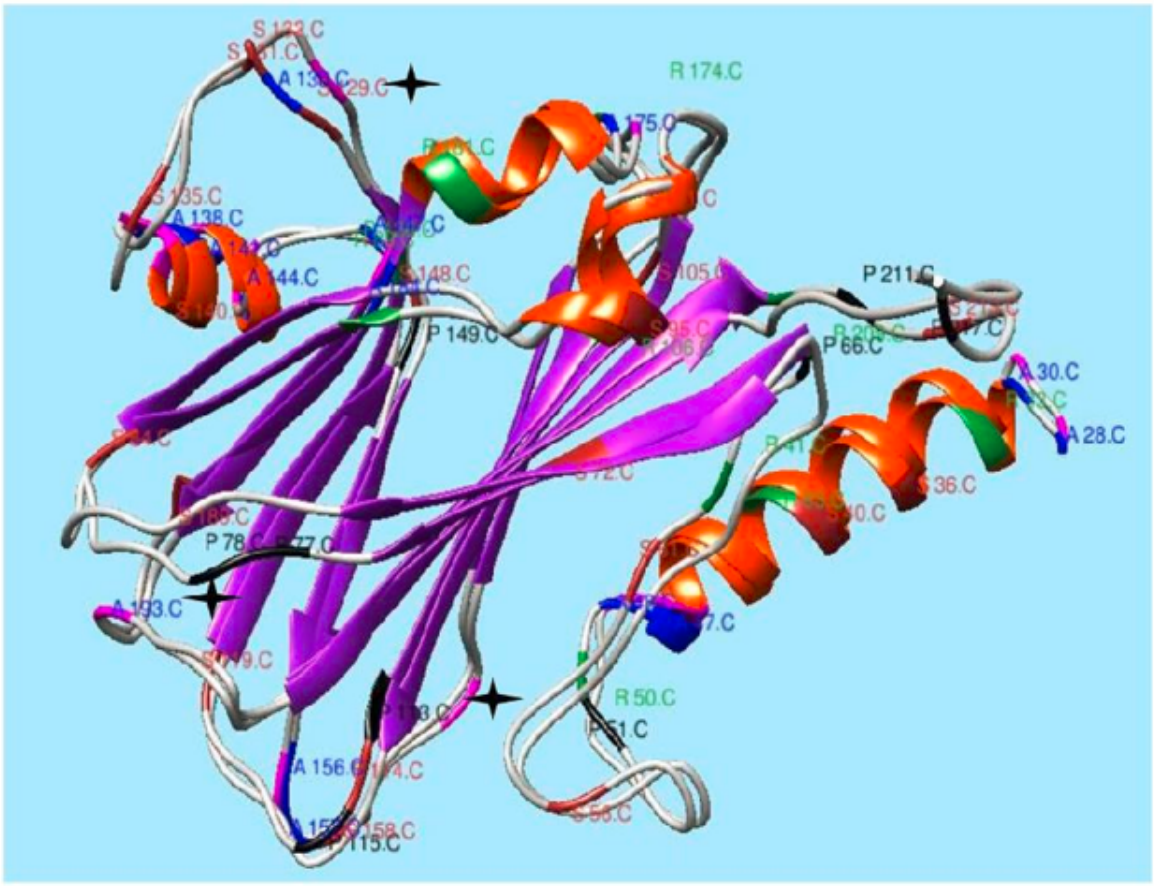

Fig. 4

Fig. 4 Superimposed coat protein model structure of chlorosis and mosaic inducing strains of CMV vindicating loop modification of βE-αEF (129 residue), βF-βG (162 residue) and βB-βC loops (78 residue). The salient mutations are presented in atomic level. The potent mutation observed on loops of coat protein was indicated with ✦mark

Figure 4

See image above for figure legend.

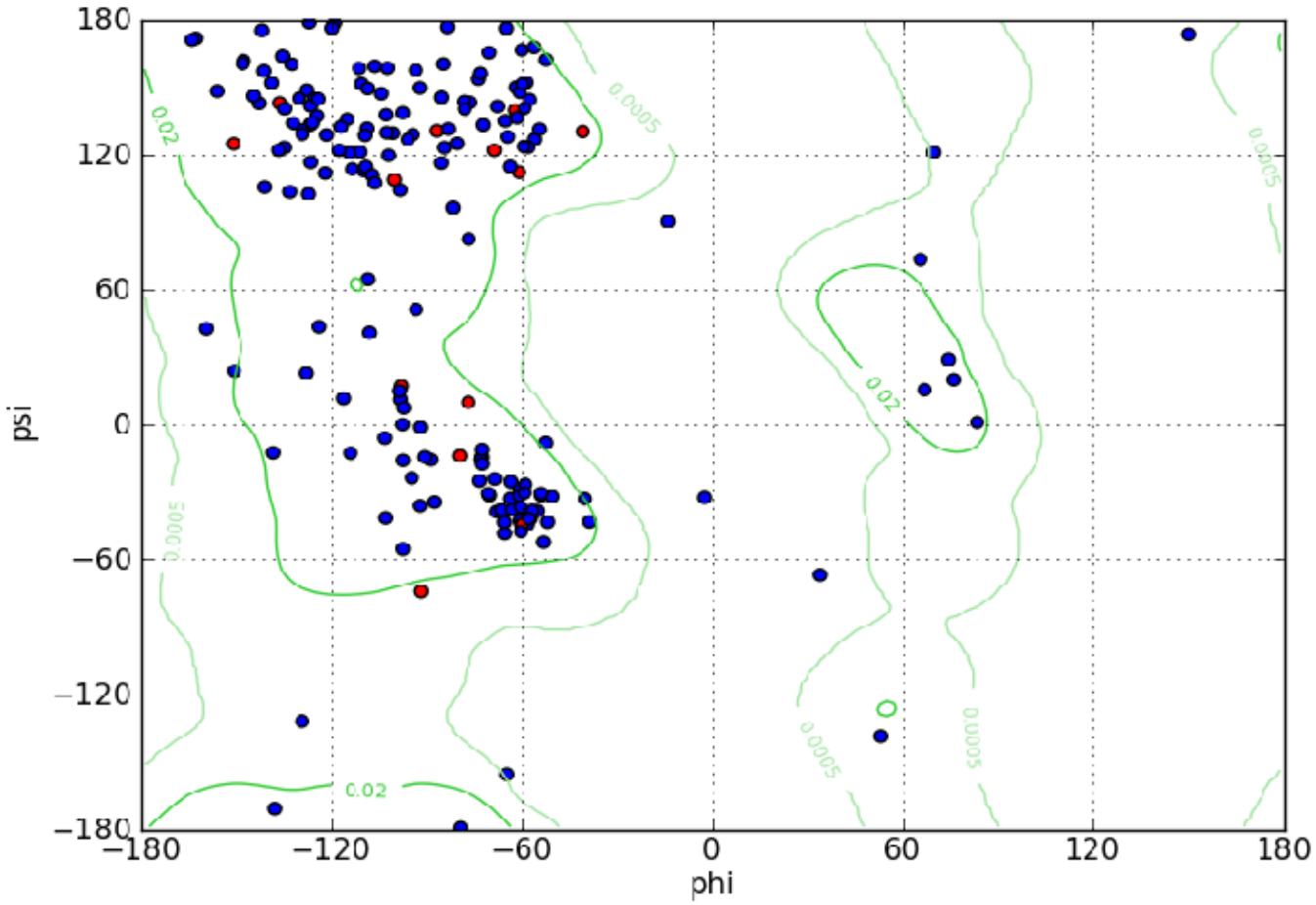

Fig. 5

Figure 5

Scoring of superimposed coat protein model of chlorosis and mosaic inducing strains showing the identical amino acids in blue color and non- similar amino acid in red color and three outliers in Ramachandaran plot. The confrontation based on the dihedral angle of peptide backbone. Moreover, phi (Φ) implies the angle of backbone atoms C-N-Cα-C and psi (ψ) indicates the angle of N-Cα-C-N atoms

Fig. 6

Figure 6

Graphical representation of comparative analysis of helix, sheets, coil and disordered protein in CP of mosaic inducing strain (a) and chlorosis inducing strain of CMV (b) using PSIPRED database. The specific regions were highlighted respectively. Pink color represents helix, yellow color represent strand, gray color represents coil and green outlined box indicates disordered proteins

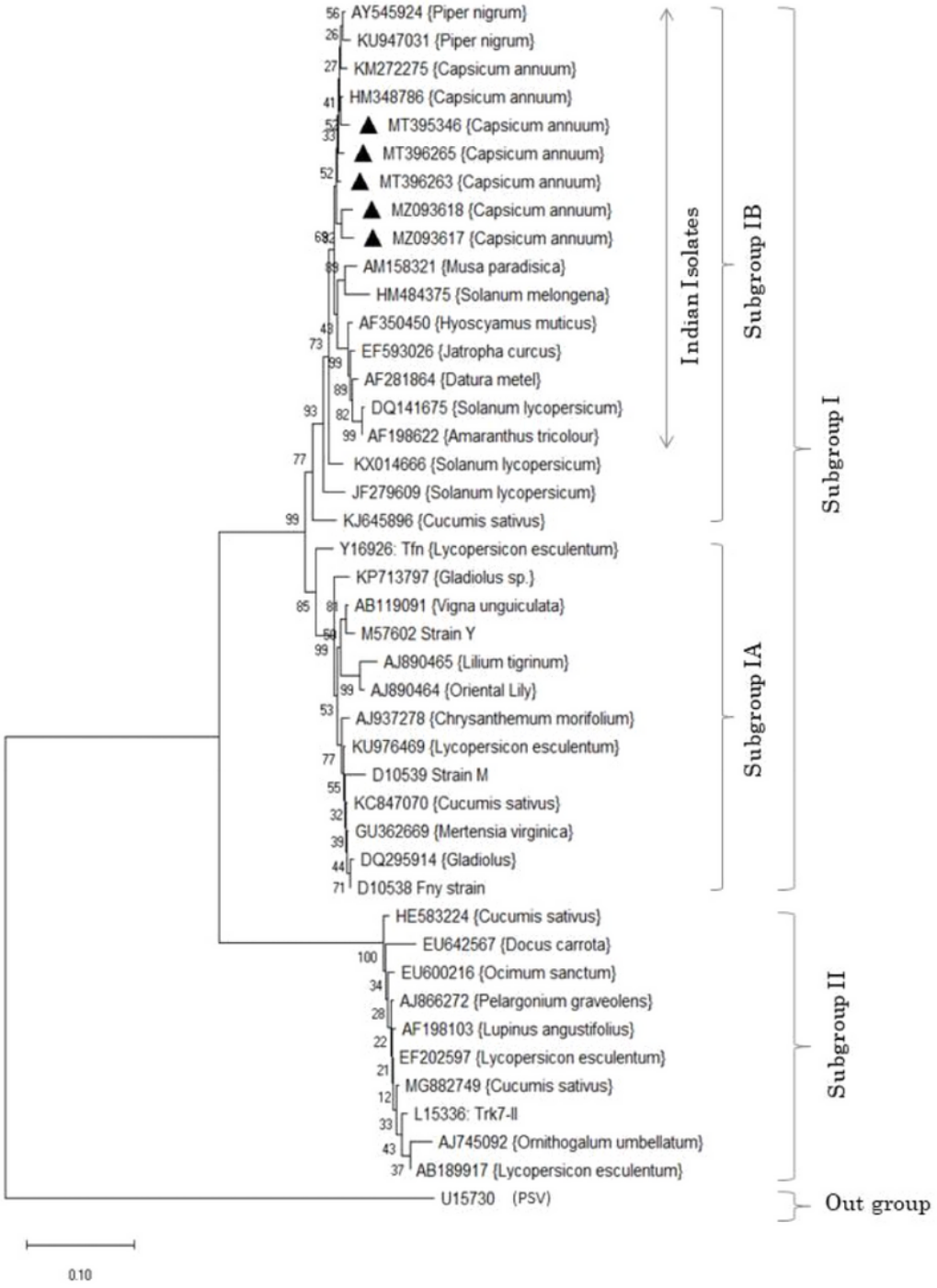

Figure 7

Coat protein based phylogenetic analysis of TN CMV isolates with other CMV isolates reported from different parts of world at amino acid level by MEGA 7.0 software using neighbor-joining method with 1000 bootstrap replication. Whereas, Fny and Tfn strains were taken as standard reference strains of subgroup IA and Trk7II strain as subgroup II reference strain. Peanut stunt virus (U15730) included as out group for rooting in phylogenetic tree

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supp.Table.doc
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