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Comparison of amino acid sequence profiles and 3-D structure prediction of Coat Protein of Sweet potato feathery mottle virus (SPFMV) reveal strain variation

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Abstract

Sweet potato feathery mottle virus (SPFMV) under Potyviridae family is the most widespread disease in sweet potato (*Ipomoea batatas L.*) across the world and causes differential symptoms of feathery mottle and degeneration of leaves and deformed storage root. The present study highlights the enhanced molecular resolution and 3-D prediction of amino acid of coat protein of seven SPFMV strains. Viral coat protein (CP) derived from an isolate (Gene bank Accession No.HM035545 and poly protein ID D6R1L4_9POTV) BCKV, India showed close relationship with RC (Russet Crack) strain and diverged from the strains 4C, EA, S, O and K1 of SPFMV. Protein Feature View of PDB entries mapped with watermelon mosaic virus (WMV) Polyprotein (PF00767) to a UniProtKB sequence S480335 predicted structural similarities for the SPFMV strains in PDB ID 5ODV for WMV. Analysis of Nuclear Localization Signal (NLSs) and its prediction of CP sequences unveiled the key amino acids in the corresponding amino acid sequences of SPFMV strains required for systemic infection, viral particle formation and insect transmission and showed typically rich in arginine and lysine residues. SPFMV, BCKV isolate revealed a significant correlation between clustering of the viruses and geographical origin and sequence variation in coat protein gene of SPFMV from different subcontinents of the world is an interesting natural mutational phenomenon compared to the conserved coat protein domain of several plant viruses instead. Thelogenetic studies of polyprotein of SPFMV, BCKV isolate showed evolutionary compatibility with other viral taxa and a motif Asp-Ala-Gly (DAG) with the nucleotide sequence GATGCGGGA (nt 31-39) was found at the N-terminal region of coat protein (CP) gene of BCKV are same to other isolates and highly conserved domain which is required for aphid transmissibility. About 20 amino acids downstream from the DAG motif, there is a potential trypsin cleavage cited that is conserved in all potyviruses.

Keywords : Sweet potato, Coat protein, SPFMV, Amino acid profile, 3-D Structure

Introduction

Sweet potato (*Ipomoea batatas*, family *Convolvulaceae*) is one of the most important and robust climate resilient crops producing edible storage root which is cultivated worldwide as a source of staple food. Sweet potato [*Ipomoea batatas* L. (Lam); *Convolvulaceae*] is an important starchy tuberous root crop grown in many tropical and subtropical regions of the world. In sub-Saharan Africa sweet potato plays a major role in providing food for the population and is the second most important root crop after cassava (Hijmans et al., 2001). Mexico and Central America are thought to constitute the centre of origin of the crop (Zhang et al., 2004). It is the seventh

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most important food crop in the world after wheat, rice, maize, potato, barley and cassava (FAO, 1993).

Till date several improved varieties of sweet potato have been developed in ICAR-AICRP on Tuber Crops under Central Tuber Crops Research Institute, Thiruvananthapuram and presently sweet potato has become one of the major crops and cultivated in commercial scale in most of the states in India. But the average productivity of sweet potato is declined due to several biotic and abiotic factors which limit the productivity of this crop worldwide. Among these, viral diseases pose significant loss of the crop in terms of yield. The most devastating virus-induced syndrome is sweet potato virus disease (SPVD). It is the most significant disease economically since diseased plants generate nearly no usable yield (Gibson et al., 1998; Karyeija et al., 1998). Although SPVD can be controlled by healthy stock programmes, phytosanitation and cultural measures, these are difficult to integrate with subsistence production system used by resource poor farmers (Gibson et al., 2004). Selection by farmers to more resistant, better performing landraces for planting has reduced SPVD incidence in the field in East Africa, resulting in improved yields (Karyeija et al., 2000, Mwanga et al., 2002), but no current cultivar grown in any part of the world is immune to SPVD. To date, twenty virus species from distinct virus families are known to infect sweet potato (Valverde et al., 2007). Among virus diseases, *sweet potato leaf curl virus* (SPLCV) and *sweet potato feathery mottle virus* (SPFMV) cause more destructive diseases especially in Africa and Asia. (Anonymous, 1992). Abad et al., (1992) and Wang et al., (2007) reported worldwide distribution of *Sweet potato feathery mottle virus* (SPFMV) and the different serotypes of SPFMV were also identified (Karyeija et al., 2000).

Sweet potato feathery mottle virus (SPFMV) belongs to the genus *Potyvirus* (family *Potyviridae*) and is found everywhere sweet potato is grown (Clark and Moyer, 1988; Moyer and Salazar, 1989; Loebenstein et al., 2004; Valverde et al., 2007). SPFMV has flexuous filamentous particles between 830-850 nm in length. Its genome consists of a single stranded, linear, positive RNA of about 10.6 kb (Sakai et al., 1997). SPFMV is transmitted in a non-persistent manner by several aphid species, including *Aphis gossypii*, *A. craccivora*, *Lipaphis erysimi*, and *Myzus persicae*. It can be transmitted mechanically to various *Ipomoea* species, although some strains have been reported to infect *Nicotiana benthamiana* and *Chenopodium* spp. (Loebenstein et al., 2004). Several strains of SPFMV have been identified based on symptoms, host range, serology, and nucleotide sequences (Moyer and Kennedy, 1978; Cali and Moyer, 1981; Kreuze et al., 2000; Wang et al., 2007). Most sweet potato cultivars infected with SPFMV alone show only mild symptoms that include vein clearing, irregular chlorotic patterns (feathering) along the leaf mid-rib, and chlorotic spots that sometimes have purple pigmented borders especially in the older leaves. Depending on sweet potato cultivars, storage roots of infected plants may show external necrosis if infected with the russet crack strain of SPFMV (Moyer and Kennedy, 1978; Clark and Moyer, 1988). Losses due to SPFMV infection are minimal, except in highly susceptible cultivars (Clark and Moyer, 1988; Karyeija et al., 1998). The ubiquitous presence of SPFMV has often masked the presence of other potyviruses. Clark et al., (2002) stated that a potyvirus complex affects sweet potatoes, but it is not clear how these potyviruses relate to one another. In the US, SPFMV is universal, but two other potyviruses, *Sweet potato virus G* (SPVG) and Sweet potato virus 2 (SPV2) are also common (Souto et al., 2003; Valverde et al., 2007). It has been reported in most tropical and sub-tropical countries as well as in the warm temperate regions (Salazar and Fuentes, 2001). Souto et al., (2003) observed that universal presence of SPFMV has often overshadowed the presence of other viruses in sweet potato, especially those belonging to the same family, such as *Ipomoea vein mosaic virus* (IVMV) and *Sweet potato virus G* (SPVG), making the effort to isolate them very difficult. Doolittle and Harter (1945) first reported *Sweet potato feathery mottle virus* from Maryland, USA. At the same time Sheffield (1957) described two viruses from East Africa, named virus A and B and the first one also called sweet potato mosaic syndrome Sinha and Tarafdar (2007).

SPFMV enters the host cell via a stylet of several aphid species (e.g *Aphis gossypii*, *Myzus persicae*) in a non-persistent manner. Their host range is narrow, limited to plants of the family *Convolvolaceae* (genus *Ipomoea*). Some strains have been reported to infect *Nicotiana benthamiana* and *Chenopodium amaranticolor* (Campbell et al., 1974; Moyer et al., 1980). As with other potyviruses, traditional criteria to discriminate between species and isolates are predominantly based on serology and biological criteria such as host range, cross protection and symptomatology (Shukla et al., 1994). Green et al., (1988) demonstrated that SPFMV can routinely be diagnosed by indexing on a sensitive indicator host *Ipomoea setosa*. Apart from Australasia and Oceania, molecular data on at least some SPFMV isolates are available from the major parts of the world where sweet potato is an important crop. The common strain (SPFMV-C) and russet crack strain (SPFMV-RC) of SPFMV were originally described based

on serological difference and the different types of symptoms induced in sweet potato (Moyer and Kennedy, 1978; Moyer et al., 1980; Cali and Moyer, 1981).

Sweet potato feathery mottle virus (SPFMV) under Potyviridae, genus *Potyvirus* is the most widespread virus infecting sweet potato and possibly occurs wherever sweet potato is grown (Brunt, 1987). Five different strains likewise Russet Crack (RC), Ordinary (O), East African (EA), K1 and C strains of SPFMV have been reported. In India, only RC strain is prevalent and cause severe damage of the tuber. Successful transmission of Potyviruses by their aphid vectors depends upon the interaction of two viral proteins: the coat protein (CP) and helper component proteinase ± HC-Protein. Potyvirus HC-Pro mediates aphid transmission through protein-protein interactions, serving as a bridge between the coat protein of virions and surfaces of the aphid maxillary food canal and foregut. The goal of this study is to comparative analysis of the features of amino acid residue and 3D- structure of coat protein of *Sweet potato feathery mottle virus* (SPFMV) BCKV isolate and other reported strains in the world with a focus on new routes for strain identification of SPFMV.

Materials and Methods

Sweet potato feathery mottle virus (SPFMV) is frequently occurring in the field gene bank of the sweet potato of the ICAR-AICRP Tuber Crops Kalyani Centre, and the leaf samples were collected from the plants showing typical symptoms (Fig. 1 a-f) of SPFMV for the study.

Extraction of total gRNA and synthesis of cDNA

Total plant genomic RNA was extracted by using RNeasy Plant Mini Kit (Qiagen, Cat No. 74903) as per the product protocol. 50 mg of infected fresh leaf sample was used in each case and extracted with RTL buffer and poured into QIAshredder spin column subjected to centrifuge for 2 min at 1000 rpm. The samples were placed on RNeasy mini column and were centrifuged at 10,000 rpm for 15sec. To elute total RNA, add $40\mu l$ of RNase-free water to the RNeasy silica-gel membrane, centrifuge at 10,000 rpm for 1 minute, and store in the $-80^{\circ}C$ freezer. C-DNA from the gRNAs of the leaf samples was synthesized using RevertAid^{TM} First Strand cDNA Synthesis Kit (Fermentas Life Sciences, Cat # K1622) following the steps according to the manufacturer's protocol. The first strand cDNA thus synthesized was directly utilized for amplification by PCR.

RT-PCR amplification and sequencing

The Reverse Transcription PCR was performed with the cDNA of the respective samples collected from the SPFMV infected plants. Predesigned degenerated Potato Virus Y specific primers pairs were used to confirm the presence of PVY (Table 1). The PVY positive cDNA samples were subjected to RT-PCR using SPFMV specific primers (Table 1) encoding the polyprotein region between nt 9828-10772 for confirmation of presence of SPFMV. The RT-PCR was performed with initial

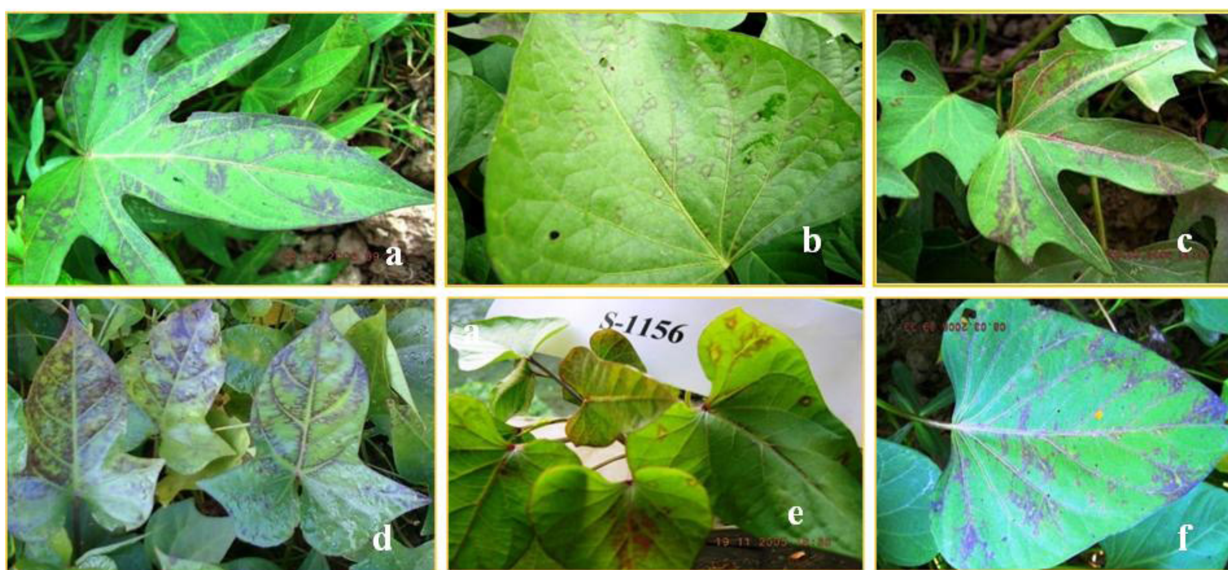

Fig. 1. Field symptoms of Sweet potato feathery mottle virus (SPFMV). (a-f) symptoms in different varieties of sweet potato

denaturation at 94°C for 3 min followed by thirty-five cycles of denaturation for 30 s at 94°C, annealing at 58°C for 60 s and elongation at 72°C for 90 s followed by a final extension at 72°C for 7 min. The amplicon of SPFMV was cloned and sequenced in both orientations. The positive clones for the fragment were sequenced commercially from Eurofins Genomics India Pvt. Ltd., Bangalore. The sequence was submitted to NCBI database for accession number. The sequenced fragment was compared with previously reported Indian and the other isolates. The cloned amplified fragment of BCKV isolate was sequenced and submitted to GeneBank as a sequence of polyprotein under the NCBI accession number HM035545.

Table 1. RT-PCR based detection of SPFMV

Primer		Sequence
POT 1	F	5' GAC TGG ATC CAT TBT CDA TRC ACC A 3'
POT 2	R	5' TGY GAY GCB GAT GGY TC 3'
POT 2-a	R	5' GAC GAA TTC TGY GAY GCB GAT GGY TC 3'
POT 5	R	5' GCA GGA TCC AAY ATH ATH GAR AAT GG 3'
SPFMV	F	5' CATCAATCTAATGAGAGTACTG 3'
SPFMV	R	5' AGTGCAGAGGATGTCCTATTG 3'

Bioinformatics and protein modelling

The gene index was assessed by self-mega blast and BLASTN program of NCBI with sequence database of SPFMV. The sequence was compared with the 35 polyproteins of *Sweet potato feathery mottle virus* (Table 2). The nucleic acid (NA) and amino acid sequences (AA) of the poly protein covering protein part was analyzed with other strains and AA was submitted with Protein data Bank (PDB); the 3D structure of the protein of the respective strains were compared. Bioinformatics- The gene index was assessed by self-mega blast and BLASTN program of NCBI with sequence database of SPFMV. EBI Clustal W, MEGA-X and the matrix were generated using the program Sequence Demarcation Tool (SDT v.1.2). Nuclear Localization Signal (NLS) was detected in cNLS Mapper and AA alignment was generated using MAFFT. Coat Protein structure was predicted using five independent prediction programs (PSI-PRED), I-TASSER, HMM, Phyre-2 and 3-D Structural synchronization and prediction of five available strains of SPFMV was compared in JSmol and EzMol Molecular

Table 2. Sweet potato feathery mottle virus accessions used for the comparison of coat protein (CP) genes among the isolates

Sl.No.	Gene Bank Acc. No.	Polyprotein ID	Strain	Isolate name	Country	Abbreviation
1	HM035545	D6R1L4_9POTV 317 aa	RC	BCKV	India	SPFMV
2	EF015398	A0FJU6_9POTV 315 aa	RC	CTCRI	India	SPFMV
3	EU021064	B1NQG3_9POTV 523 aa	RC	M2-41	Peru-Canete	SPFMV
4	D38543	Q88274_9POTV 340 aa	--	Severe	Japan	SPFMV
5	AJ310201	Q80MW6_9POTV 530 aa	RC	Jinan	China	SPFMV
6	AJ515379	Q8AYT5_9POTV 525 aa	--	Egypt 9	Egypt	SPFMV
7	EU021065	B1NQG4_9POTV 523 aa	RC	Fio	Peru-Canete	SPFMV
8	AM050889	Q0W9C0_9POTV 530 aa	RC	Aus120-7	Australia	SPFMV
9	EU809482	D2CTJ6_9POTV 516 aa	RC	OR1-1	French Polynesia	SPFMV
10	EU809506	D2CTM0_9POTV 370 aa	RC	NZ4-2	New Zealand	SPFMV
11	AJ781777	Q59A01_9POTV 530 aa	RC	Aus-6	WesternAustralia	SPFMV
12	AJ781776	Q59A02_9POTV 530 aa	RC	Aus5	Australia	SPFMV
13	EU021066	B1NQG5_9POTV 523 aa	RC	Kmt mil	Peru Cenate	SPFMV
14	AJ781787	Q599Z1_9POTV 530 aa	--	Apa	Uganda: Apachi	SPFMV
15	AJ781789	Q599Y9_9POTV 530 aa	--	Mpg2	Uganda Mpig	SPFMV
16	AAS99562	Q5GIV1_9POTV 523 aa	O	115/1s	Kenya	SPFMV
17	EU809489	D2CTK3_9POTV 370 aa	RC	OR2-1	French Polynesia	SPFMV

18	AY459602	Q5QIX6_9POTV	315 aa	RC	XN3	China	SPFMV
19	AM050890	Q0W9B9_9POTV	377 aa	RC	Aus142A	Aus: E. Kimberley	SPFMV
20	AJ781775	Q59A03_9POTV	530 aa	C	Aus2	Australia-Western	SPFMV
21	AJ515378	Q8AYT6_9POTV	525 aa	C	Egypt1	Egypt	SPFMV
22	AF015540	O37015_9POTV	315 aa	K1	Korean	Korea	SPFMV
23	D86371	O39734_9POTV	493 aa	S	Tarumi		SPFMV
24	AJ310202	Q80MW5_9POTV	530 aa	--	Hangzhou	China	SPFMV
25	EU809505	D2CTL9_9POTV	370 aa	RC	NZ4-1	New Zealand	SPFMV
26	EU809491	D2CTK5_9POTV	370 aa	RC	OR2-3	French Polynesia	SPFMV
27	EU809507	D2CTM1_9POTV	370 aa	RC	NZ4-3	New Zealand	SPFMV
28	AJ539130	Q80MW3_9POTV	530 aa	EA	Bny	Uganda	SPFMV
29	AY459600	Q5QIX8_9POTV	519 aa	EA	Canary3	Canary island	SPFMV
30	AY459599	Q5QIX9_9POTV	356 aa	EA	Portugal	Portugal	SPFMV
31	AY459595	Q5QIY3_9POTV	356 aa	O	Arua	Uganda	SPFMV
32	AY459598	Q5QIY0_9POTV	356 aa	O	Tz4	Tanzania	SPFMV
33	U96624	O10648_9POTV	315 aa	O	Strain5	Argentina	SPFMV
34	AJ781778	Q59A00_9POTV	527 aa	C	Aus4	W-Australia	SPFMV
35	AJ781779	Q599Z9_9POTV	527 aa	C	Aus5c	W-Australia	SPFMV

display wizard, the online platforms for protein structure and function predictions using homology modeling.

Results and Discussion

Detection of *Sweet potato feathery mottle virus* (SPFMV) in sweet potato plant

SPFMV suspected plants of sweet potato were used for detection of SPFMV by Reverse Transcriptase (RT) - PCR method. Four primer pairs (SPFMV F/R, POT1/POT2, POT2a and POT5) were used for the detection of SPFMV by Reverse Transcription PCR (RT-PCR). A fragment ~518bp was amplified by the degenerate primer POT1/POT2a, band size of ~550bp was amplified with the primers pair POT1/POT2 and ~490bp amplicon was produced by the primer pair POT1/POT5 (Fig. 2a) which confirmed the presence of Potyvirus in sweet potato. Whereas coat protein gene specific primer of SPFMV amplified fragment of ~954bp (Fig. 2a). Further appearance of ~954bp band confirmed the infection of SPFMV in the suspected sweet potato plants (Fig. 2b). The results clearly indicated that SPFMV suspected plants amplifying~954bp band had

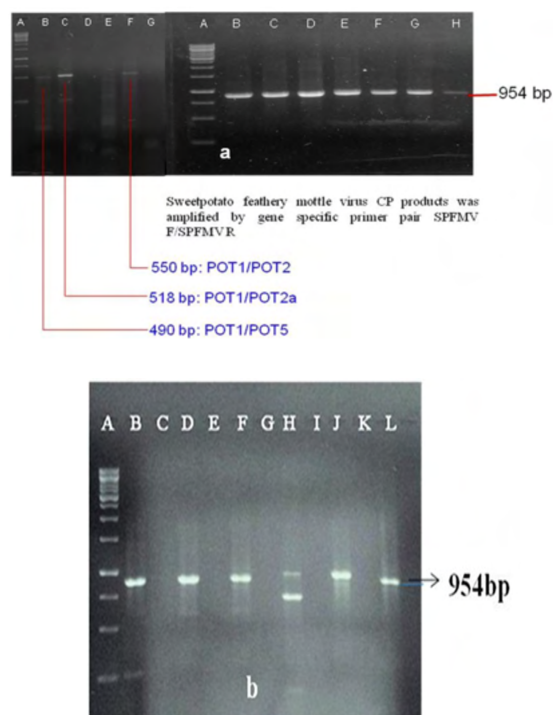

Fig.2. Amplification of the bands of the SPFMV (Potyvirus) using Forward primer POT1 and POT2,

POT2a, POT5 reverse primers and SPFMV gene specific primer. Amplification of 550bp, 518bp and 490bp bands of cDNA of the diseased samples of sweet potato using Potato Virus Y specific degenerated primer Fragment (~954bp) of partial polyprotein covering sub-genomic RNA of Coat protein gene of SPFMV, BCKV isolate

infection with SPFMV. Souto (2003) showed same size band (450bp) amplification by using primer pair POT1/ POT2a. Sinha and Tarafdar (2007) found similar size of DNA amplification by RT-PCR using primer pairs POT1/ POT2, POT1/POT2a. Colinet et al., (1998) revealed combined assay of reverse transcription and polymerase chain reaction (PCR) utilizing degenerate genus-specific primers POT1/ POT2 and found respected fragment.

Amino acid sequence analysis of coat protein of SPFMV and protein modeling

The sequenced fragment of cDNA clone of BCKV isolate of SPFMV was submitted to GeneBank as a partial sequence of polyprotein under the NCBI accession number HM035545 (BCKV isolate). The AA sequence was compared with the 35 polyproteins of *Sweet potato feathery mottle virus* retrieved from NCBI. Pairwise matrix of amino acid sequence of coat protein (CP) gene shared 79-96% amino acid sequence identity with other accessions. Highest amino acid identity (96%) was observed with most of the coat protein gene of *Sweet potato feathery mottle virus* (SPFMV) isolates viz. Kerala (India), Japan, Egypt 9 (Egypt), XN3 (China), Korean 1 (Korea) and Tarumi (Japan) followed by 95% identity with the isolates M2-4 (Peru-Cenate), Jinan (China), Fio (Peru-Cenate), Aus120-7 (Australia), OR1-1 (French-Polynesia), Aus6 (Western-Australia), Aus5 (Australia), Katmil (Peru-Cenate), Mpg2 (Uganda), OR2-1 (French-Polynesia), Aus2 (Western-Australia), NZ4-1 (New Zealand), OR2-3 (French-Polynesia) in Table 2. The SPFMV of most of the countries were found to be 'RC' strain, 'EA', 'O' strains are prevalent in African and European countries, 'C' strain is available in Australia and Egypt whereas K1 strain is confined in South Korea. Highest disparity was showed by the SPFMV strains 'C' followed by CP of 'O' strains. Similar results were found by the phylogenetic analysis tree constructed by CLUSTAL W Program version 2.0 based on CP gene amino acid sequence. Phylogenetic neighbor joining tree revealed that all coat protein genes of *sweet potato feathery mottle virus* isolates were grouped into four major clusters. CP gene from BCKV and CTCRI, Indian isolate was found in the cluster I, but it was further classified in to two groups, CP from BCKV, CTCRI, Egypt1, Egypt9 and Aus142-A were found in the sub-cluster I and cluster II was formed with RC strain of *Sweet potato feathery mottle virus* coat protein. SPFMV strain C was found to group the cluster IV that was distinct from the cluster I, which revealed that *Sweet potato feathery mottle virus* West Bengal isolate is distinct type from group for. All the strains O were clustered in group III and strain EA was found in cluster II (Fig. 3). Nucleotide Sequences of five SPFMV strains of different countries were compared among the strains and with *Watermelon Mosaic virus* as out group. Interestingly, the 4C strain of Australia and O strain of Argentina were found highly diverged and only 61% na similarity and 85-88% na identity with rest of the reported strains of SPFMV (Fig. 4) when compared with *Watermelon Mosaic Virus* (*Potyvirus*).

A motif Asp-Ala-Gly (DAG) with the nucleotide sequence GATGCGGGA (nt 31-39) was found at the N-terminal region of coat protein (CP) gene of BCKV isolate at the position of amino acid 11-13 in the sequence (Fig. 5). Clustering pattern of the isolates may provide indication

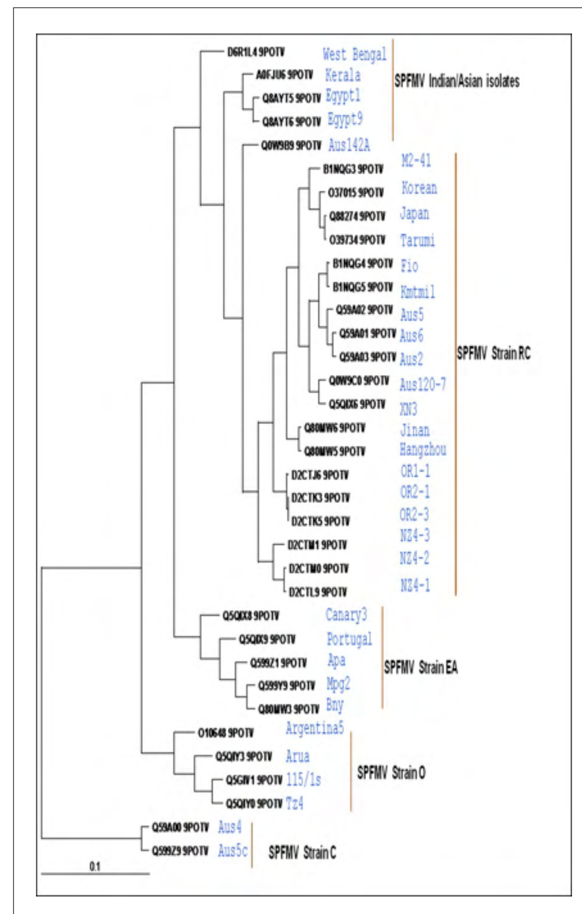

Fig. 3. Neighbour joining tree analysis of CP gene of SPFMV West Bengal isolate. (Tree was constructed by CLUSTAL W program version 2.0 online ebi.uk.com)

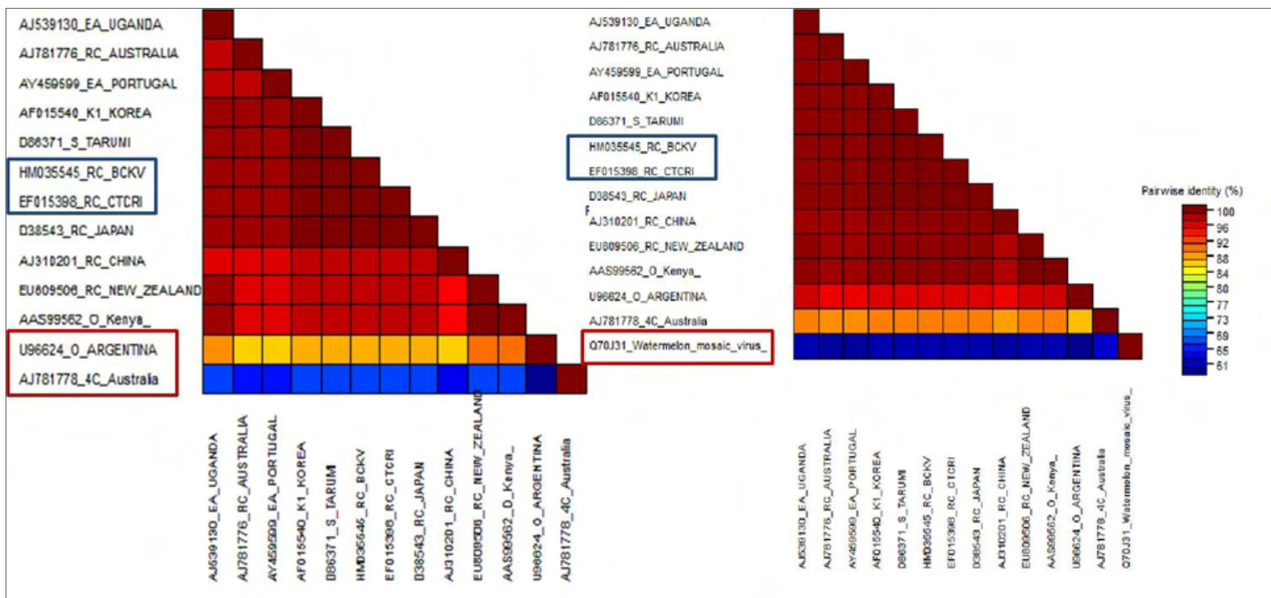

Fig. 4. Colour-coded pairwise identity matrix generated from the nucleotide Sequence Demarcation Tool (SDT v.1.2). Each coloured cell represents a percentage identity score for two sequences. Colored keys indicate the correspondence between pairwise identities and the colours displayed in the matrix

of the results of the introduction of virus isolates from one geographical locality to another (Gibson and Aritua, 2002). Atreya et al., (1992) suggested that the conserved DAG motif common for aphid-transmitted potyviruses and CP size which is the third largest of all known potyviruses infecting sweet potatoes. Later Shukla et al., (1994) also confirmed that the N-terminal DAG motif required for aphid-transmissibility of potyviruses, and which is normally present close to the 5' end to coat protein and also simplified the determination of the correct position of the CP N-terminus in aphid-transmitted viruses. Potyviruses-dependent aphid transmissibility can be conferred PVX, a non-aphid-borne potexvirus, by substituting this domain for the N-terminal part of its coat protein and suggested that virus particles are released by tryptic-like cleavage of the coat protein sequence on the carboxyl side of the DAG motif (López-Moya et al., 1999). About 20 amino acids downstream from the DAG motif, there is a potential trypsin cleavage cited that is conserved in all potyviruses. However, in our present investigation of phylogeny of CP gene of derived coat protein (CP) gene of SPFMV revealed that BCKV isolate of SPFMV is very closely (79-96%) related to other reported isolates but distantly related with SPFMV-C strain The previous reports also supported our findings that DAG motif of BCKV isolate, are similar to other isolates and are highly conserved domain which is required for aphid transmissibility (López-Moya et al., 1999, Elijah et al., 2017). The N-terminus region of CP amino acid 67-68 was revealed to be TE, which encodes threonine-gutamic acid, whereas threonine is replaced by lysine in the majority of SPFMV coat protein genes. Interestingly, CP gene of SPFMV strain C revealed the deletion of those two amino acids. Classic NLS of CP is typically rich in lysine (K) and arginine (R) in all the strains of SPFMV (Fig. 5b).

The critical scrutiny of the amino acids of coat protein gene SPFMV BCKV and other strains showed a stretch of twenty nine AA (LKNAKNRLFGLDGNVSTQEEDTERHTTTD) that have been predicted as nuclear localization signal through cNLS Mapper (Kosugi et al., 2008) with the average score 2.4-2.7. The Lysine and Arginine residues for NLS sequence of coat protein gene of all the isolates was found to be 2:2 ratio except for the 4C, O and EA strains, where K was substituted by H, R and R residues respectively. The architecture of NLS was typically rich in basic amino acids such as lysine (K) and arginine (R) in all the strains of SPFMV. NLS score predicts the function in different classes of importin-α/β pathway-specific NLS (Kumar et al., 2012) and it was reported that N-terminal NLS of TYLCV CP binds to karyopherin α1 for nucleocytoplasmic trafficking (Kunik et al., 1999). However, score <5 predicted NLSs confirmed that the replication of SPFMV polyprotein is exclusively localized to the cytoplasm. Several other potyviruses which have motifs other than DAG are aphid-transmissible. López-Moya et al., (1999) revealed that creation of these motifs in TVMV through site-directed mutagenesis failed to

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Egypt-9          Q8AYT5_9POTV   .....DAG........KD.........V........A.............PESY..... 272
Egypt-1          Q8AYT6_9POTV   .....DAG........KD.........V........A.............PESY..... 272
Apachi           Q599Z1_9POTV   .....DAG........KDV........I........A..K.........V.PESY..... 277
Mpg-2            Q599Y9_9POTV   .....DAG........K.V........I........E..K.........V.PESY..... 277
NZ4-2            D2CTM0_9POTV   .....DAG.D.T....K..........I........A.............PESY..... 117
NZ 4-1           D2CTL9_9POTV   .....DAG.D.T....K..........I........A.............PESY..... 117
NZ 4-3           D2CTM1_9POTV   .....DAG.......SKIN........IV.......A..K..........PESY..... 117
Fio Peru-Cenate  B1NQG4_9POTV   .....DAG.D......K......I...V........A.............PESY....G 270
Kmt mil          B1NQG5_9POTV   .....DAG.D......K......I...V........A.............PESY....G 270
M 2-41           B1NQG3_9POTV   .....DAG.D.L....K..........V........A............TPESY..... 270
Jinan            Q80MW6_9POTV   .....DAG...S....KD.........V........A.............PESY..... 277
Hangzhou         Q80MW5_9POTV   .....DAG...S....KD.........V........A.............PESY..... 277
Aus -6           Q59A01_9POTV   .....DAG........KD.........V........A.............PESY..... 277
Aus-2            Q59A03_9POTV   .....DAG........KD.........V........A.............PESY..... 277
Aus 142-A        Q0W9B9_9POTV   .....DAG........KD.........V........A...........I.PESY..... 124
Aus-5            Q59A02_9POTV   .....DAG........KD.........V........A.............PESY..... 277
Aus 120-7        Q0W9C0_9POTV   .....NAG.D......KD.........V........A.............PESY..... 277
XN 3             Q5QIX6_9POTV   .....DAG.D......KD.........V........A.............PESY..... 62
OR 2-1           D2CTK3_9POTV   .....DAG.D......K..........V........A..K..........PESY..... 117
OR 2-3           D2CTK5_9POTV   .....DAG.D......K..........V........A..K..........PESY..... 117
OR 1-1           D2CTJ6_9POTV   .....DAG.D......K..........V........A..K..........PESY..... 263
Japan            Q88274_9POTV   .....DAG...................V........A.............PESY..... 1087
Tarumi           O39734_9POTV   .....DAG...................V........A.............PESY..... 3240
Korean           O37015_9POTV   .....DAG......A.K..........V........AALK..........PESY..... 62
West Bengal      D6R1L4_9POTV   ERTEFKDAGANPPAPKPQNIPPPPTITEGTDPEDPKQPALRAARAKQPATISIIMGRDTS 64
Kerala           A0FJU6_9POTV   .....DAG........KDV........V.....LN.A.............PESY..... 62
115/1s           Q5GIV1_9POTV   .K...DAGV......SNIN........VV.......A..K..........PESY..... 270
                                *:****:**.:* ** .:  **** *** .**** :* **:********  .   ****.
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(a)

SPFMV CP Strain	Predicted NLS	NLS Score
RC BCKV	LKNAKNRLFGLDGNVSTQEEDTERHTTTD--	2.7
4C Australia	LKNAHNRLFGLDGNVSTQEEDTERHTATD--	2.6
RC Japan	LKNAKNRLFGLDGNVSTQEEDTERHTTTD--	2.7
K1 Korea	LKNAKNRLFGLDGNVSTQEEDTERHTTTD--	2.7
S Tarumi	LKNAKNRLFGLDGNVSTQEEDTERHTTTD--	2.7
O Argentina	LKNARNRLFGLDGNVSTQEEDTERHTTTD--	2.4
EA Uganda	LKNARNRLFGLDGNVSTQEEDTERHTTTD--	2.4
	****.**********************:**	

(b)

Fig. 5. (a) Amino acid alignment of the coat protein gene of Sweet potato feathery mottle virus (SPFMV) Bengal isolate (HM035545) with related isolates of SPFMV. Within the brown box an important motif is highlighted as DAG (Aspartic acid-alanine-glutamic acid) motif which regulates the aphid transmissibility of SPFMV and conserved in all the isolates (b) Classic NLS of CP is typically rich in lysine (K) and arginine (R), the counts <5 predicted NLSs is exclusively localized to the cytoplasm

render TVMV aphid-transmissible from infected plants and suggested that the mere presence of a DAG motif does not guarantee transmissibility and that the context in which the DAG or equivalent motif is found plays a role in the process. First comprehensive report on the significance of DAG motif in *Ipomovirus* genomes was determined and the presence of this motif suggests that aphids could potentially be a vector of Cassava Brown Steak Virus (CBSV), *Squash vein yellowing virus* (SqVYV), *Coccinia mottle virus* (CocMoV) (Elijah Ateka et al., 2017). Recent reviews present the current knowledge of PVY transmission and role of DAG and other motifs for aphid transmissibility of Potyviridae (Bhoi et al., 2022). It is mentioned that certain genera of Potyviridae like Rymovirus, Poacevirus, and tritimovirus are not transmitted by aphids due to a lack of suitable amino acid motifs for proper binding (Wylie et al., 2017). It is also reported that *Rose yellow mosaic virus* (RoYMV) from the monotypic genus Roymovirus was reported to lack the DAG motif in the CP, and the substituted HC-Pro motifs PTK and KITC by the C-2x-C motif at the N-terminus, favors transmission by the eriophyid mite and *Bellflower venial mottle virus* (BVMV), the DTG motif similar to DAG is found near the N terminus of CP, but it lacks the PTK and KITC motifs, so it is non-transmissible by the aphid vectors (Wylie et al., 2017).

The Protein Data bank file of CP gene of the SPFMV was generated using PSI-PRED and the Protein 3D structure was generated using EzMol Molecular display wizard (Fig. 6a) where yellow surface color and red chemical structure was found the DAG motif of CP. The amino acid (AA) sequences of the poly protein covering Coat Protein of RC, O, 4C, EA and S strains were analyzed with reference Protein Data Bank No. 5ODV format which provides a standard representation for macromolecular structure of *Watermelon mosaic virus* (IPR001592 Poty_coat protein); the 3D structure of the protein of the respective strains were compared. SPFMV BCKV RC strain was compared with other type strains and bootstrapped with Watermelon Mosaic (WMMV), potyvirus gave clear evidence on the variation of SPFMV CP due to natural mutation. Interestingly, enhanced molecular resolution of SPFMV revealed a significant correlation between clustering of the viruses and geographical origin. This high degree of conservation in DAG motif and variation in amino acids is surprising given the differences in biological and physical properties of the SPFMV coat protein. Our data indicate that strains of the same SPFMV or different strains from different geographical locations show significant difference in amino acid sequence trans membrane topology and protein folding (Fig. 6b and c) suggesting that the strains of SPFMV posses considerable differences in the biological/physical properties of a virus and can be brought about by either one or a few nucleotide/amino acid alterations.

The AA backbone of the strains were superimposed in online version of JSmol and EzMol and it showed changes in AA among strains of different countries (Fig. 7a) but structurally same when compared with WMMV and BCKV-RC strain (Fig 7b). The AA of six available strains of SPFMV including BCKV strain were annotated in PSI-PRED for generating the PDB files and predicted in 3D annotation grid of CP sequence of SPFMV strains which predicted that the polypeptide chain could often fold into one or more distinct domains or substructures with

variable helical secondary structure (Fig. 6b). The PDB data of six strains (00d531A to 00d536A) were further accessed in secondary structure prediction in JSmol (Fig. 6c) which exposed the basic units of folding, function and evolution often have similar chain topologies of the strains with different geographical regions. Maina et al., (2018) reported the detail insight of the SPFMV and SPVC (Sweet potato Virus C) and coat protein (CP) genes phylogenetic position and strains differentiation. East Timorese sequences were within major phylogroup A's minor phylogroups EA(I) or O(II), whereas Australian sequences were within minor phylogroup O(II) or major phylogroup B(RC). It suggests at least two SPFMV and three SPVC introductions to Australia since agriculture commenced 228 years ago. With SPFMV, evidence of genetic connectivity between Australian and East Timorese sequences was found within major phylogroup A's minor phylogroup O(II). However, within this same minor phylogroup, there was also a similar genetic match between Australian and single Argentinean and Japanese

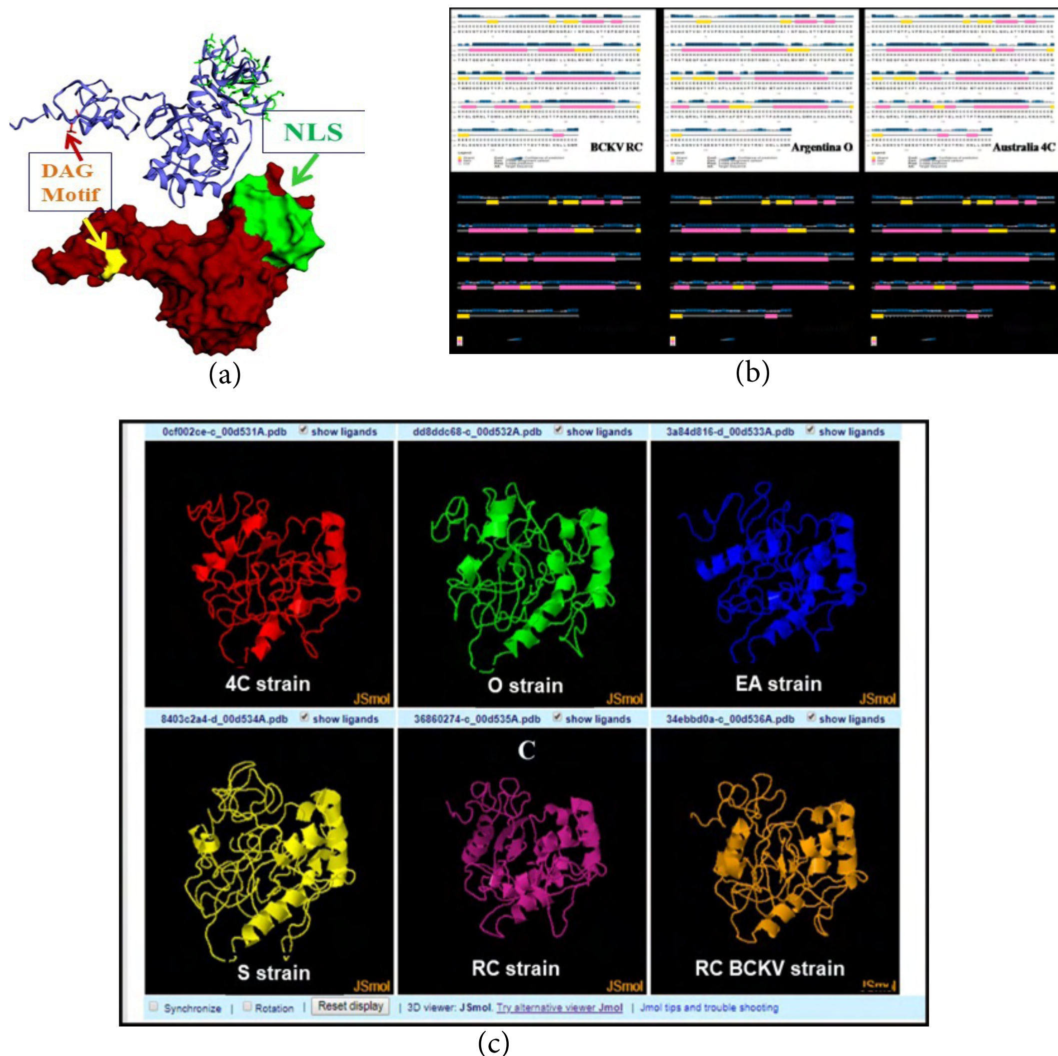

Fig. 6. Protein Feature View of PDB entries mapped to a UniProtKB sequence S480335 Model dimensions (Å) of CP subunit of SPFMV: X:72.461Y:57.702 Z:82.129; The 3-D independent prediction of the CP of six strains of SPFMV using the programms (PSI-PRED), Psipred, I-TASSER, HMM, and Phyre-2 online platforms ion. (a) Protein 3D structure of SPFMV coat protein generated using Phyre2 and the DAG motif is shown with chemical structures using EzMol Molecular display wizard, (b) Predicted 3-state annotation grid of CP sequence of SPFMV strains show the polypeptide chain could often fold into one or more distinct domains or substructures considered as the basic units of folding, function and evolution often have similar chain topologies and (c) 3-D Structural prediction (JSmol) of six available strains of SPFMV predicted variation in the secondary structure of the protein

sequences. Parrella et al. (2006) analyzed the Italian isolate of SPFMV based on both the predicted size of the putative coat protein-encoding region (939 nucleotides) and first time reported that the Italian isolate of SPFMV belongs to SPFMV subgroup C strain.

Though SPFMV alone generally causes only minor damage to sweet potato cultivars, the RC strain is associated with russet cracking of the tuberous roots in certain cultivars and has been reported from USA (Cali and Moyer, 1981), Japan (Sakai et al., 1997), Korea (Ryu et al., 1998), China (Chen et al., 2001) and from Egypt (Ishak et al., 2003). Isolates of strain C deviate from RC by 82% amino acids and have been reported from Argentina, China and USA (Abad et al., 1992; Colinet et al., 1998). Phylogenetic analysis of the nucleotide (nt) sequence of the CP-encoding regions not only distinguished C from RC readily, but also revealed two additional phylogenetic lineages, the O and EA strain groups (Kreueze et al., 2000). RC, O and EA are closely related to each other but are phylogenetically distant from C. Strain EA has a much more restricted geographical distribution from others (Kreueze et al., 2000; Mukasa et al., 2003; Souto et al., 2003).

Maina et al., (2018) also reported that two major phylogroups (A and B = RC) and two minor phylogroups (EA[I] and O[II]) within A; East Timorese sequences were in EA(I) and O(II), whereas Australian sequences were in O(II) and B(RC) and suggested that SPVC, CP is prevailed in sufficient diversity to distinguish major phylogroups A and B and six minor phylogroups within A (I to VI); East Timorese sequences were in minor phylogroup I, whereas Australian sequences were in minor and major phylogroups II and VI and B. Maina et al., (2018) strongly suggested that at least two (SPFMV) and three (SPVC) separate introductions to Australia where Aus4C and New Zealand isolate NZ4-4 has close identity in nucleic acid and reported that sweet potato plantings in the Australian continent and neighbouring Southeast Asia by which at least two (SPFMV) and three (SPVC) separate strains introduced. Utilizing the most advanced bioinformatics, coupled with high performance computing alignment visualization tools, we have uncovered pattern of strain variation in SPFMV based on coat protein topology and also enlighten the evolutionary history of the presence of the DAG motif in SPFMV linked to aphid transmission.

The present study also emphasized the molecular characterization of the coat protein of SPFMV. 3D protein structure prediction and bioinformatics can open up new ways to culminate virus strain identification as well as vector-mediated virus transmission. It is concluded that SPFMV, BCKV RC strain is highly diverse compared to C, O, EA and other RC strains but closely related to the RC strains of CTCRI-India, Egypt and Australia. PDB entry (50DV) of CP of BCKV RC strain, proved to be under Potyviridae superfamily with the basic backbone of the CP with changes of amino acids in several strains. Further, natural mutational sensitivity and amino acid changes in

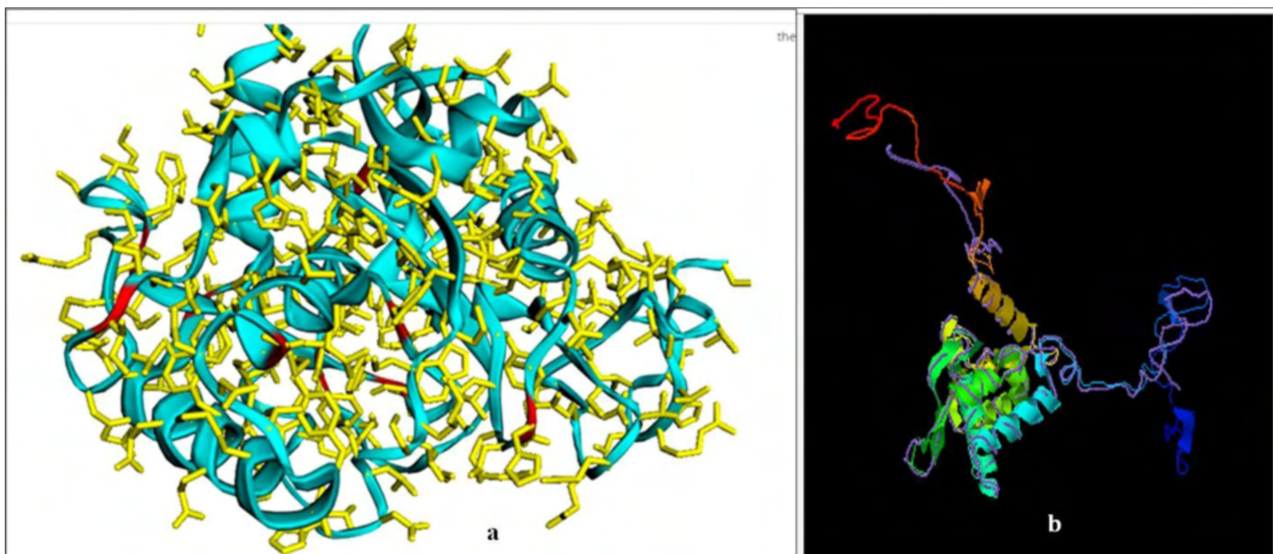

Fig. 7. Superimposed predicted sheet of CP of SPFMV strains in EzMol, Molecular display wizard.html. (a) Basic backbone of the coat protein (Blue), changes of Amino acids in several strains (Red) in natural mutation in different geographical areas and (b) 3DStructure of WMMV CP pfam00767 is the only member of the superfamily cl02961 superimposed on SPFMV BCKV RC strain

the coat protein of SPFMV reveals strain variation but motif for aphid transmissibility is conserved.

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