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Jadhav PR
Department of Plant Biotechnology, College of Agriculture, Vellayani, Kerala, India

Soni KB
Department of Plant Biotechnology, College of Agriculture, Vellayani, Kerala, India

Sona Charles
Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, Kerala, India

Swapna Alex
Department of Plant Biotechnology, College of Agriculture, Vellayani, Kerala, India

Correspondence
Jadhav PR
Department of Plant Biotechnology, College of Agriculture, Vellayani, Kerala, India

An *in silico* phylogenetic analysis and homology modelling of coat protein of banana bract mosaic virus

Jadhav PR, Soni KB, Sona Charles and Swapna Alex

Abstract
The present study was undertaken to enable the development of molecular strategies against BBrMV based on coat protein (CP) gene. The CP gene of Banana Bract Mosaic Virus (BBrMV) from infected Musa sp. var. Grand Naine (Kerala) was isolated and analysed using the *BLAST* tool. The fifty-six sequences showing 94-98% sequence similarity with this sequence were retrieved from Gen Bank database for divergence study. The phylogenetic analysis divided the isolates into two major clades with mean distance of 0.030. A conserved region of 234 nucleotides was identified in all the isolates. Neutrality test and secondary structure prediction of CP gene suggested purifying selection. The CP of all isolates showed the presence of conserved DAG motif while rad4 which functions in recombination and repair was found only in CP of isolate from Grand Naine (Kerala). CP structure was modelled using Modeller, I-tasser and swiss-modeller.

Keywords: Coat protein, banana bract mosaic virus, grand Naine, phylogenetic analysis, structural analysis, homology modelling

Introduction
Banana Bract Mosaic Virus (BBrMV), a flexuous filamentous pot virus with positive-sense ssRNA of 10kb genome size, is one of the important viruses affecting banana worldwide causing a serious damage and yield reduction to about 30-70% [1-3]. Its epidemic is controlled by phyto sanitary measures and use of virus free planting material [4]. The chemical control strategies used against aphid vectors (*Pentalonia nigronervosa, Aphis gossypi and Rhopalosiphum maidis*) are not efficient and fast enough to prevent non-persistent transmission of virus. The non-availability of BBrMV resistant source and the sterile triploid nature of popular banana cultivars makes it difficult to develop resistant varieties.
The advanced technologies used against viruses include RNAi mediated gene silencing [5-7] and CP mediated virus suppression [8-11]. These technologies demand the nucleotide sequence information of the target genes, especially the level of variability of the virus genes targeted [12-17]. Variations are very common in viruses which make them resistant to the defence mechanisms developed by the host. Since coat protein is an important target for development of resistance, this study was undertaken to obtain sequence and structural information on CP gene of BBrMV infecting Grand Naine variety of banana. Evolutionary divergence of the virus was predicted and homology modelling was performed to predict the structure of CP.

Materials and methods
Isolation of CP gene and analysis of sequence
Banana plants (var. Grand Naine) showing symptoms of BBrMV were collected from the field of College of Agriculture, Vellayani, Kerala and total RNA was isolated [18]. The cDNA was synthesised using Thermo Scientific Verso cDNA Synthesis Kit and used to amplify the coat protein gene. The forward primer (TCTGGAACGGAGTCAACCA) and reverse primer (TATCACGCTTCACATCTTCA) were designed based on the sequences of Trichy isolate of BBrMV available in Gen Bank. The thermal profile used for amplification was 94ºC for 5 min (initial denaturation), 35 cycles at 94ºC for 30s (denaturation), 58.8ºC for 30s (annealing), 72ºC for 45s (extension), and 72ºC for 5 min (final extension). The amplified PCR product was sequenced, analysed with Basic Local Alignment Search Tool (*BLAST*) and deposited in Gen Bank database. Conserved region in the sequences was identified using Dna SP v.6.

Phylogenetic analysis and tree construction

Phylogenetic analysis was conducted using Maximum Composite Likelihood model in MEGA7 (Molecular Evolutionary Genetics Analysis Version 7.0) software [19]. The robustness of the lineages in the phylogenetic tree was analyzed by bootstrapping using 1000 resampling and evolutionary divergence of 57 BBrMV isolates was calculated using MEGA7 software.

Test of neutrality

DNA Sequence Polymorphism version 6 (Dna SP v.6) [20] was used for Tajima's D test of neutrality [21].

Secondary structure prediction

The helix, sheet, and turn of amino acid sequence of CP of BBrMV was predicted by PSI-blast based secondary structure prediction (PSIPRED) [22] and Chou and Fasman secondary structure prediction (CFSSP) server [23]. Motif in the coat protein gene were searched using Motif Alignment & Search Tool (MAST) of MEME suite 5.0.1[24].

Homology modelling of coat-protein and evaluation

CP structure of BBrMV was predicted through comparative modelling using Modeller 9v9 [25], I-tasser [26], and Swiss-Model [27] by picking the most suited template. Predicted structure of coat protein of BBrMV was evaluated using SAVES v5.0 server.

Results

Isolation of CP gene and analysis of sequence

RT-PCR yielded an amplicon of about 845 bp which was deposited in Gen Bank database with accession number MH253466. BLASTn result showed a maximum of 98% sequence identity and E-value zero with the isolate from cardamom (Kerala and Karnataka) and isolate from Red Banana (Tamil Nadu). Nucleotide sequences of isolates from various regions of India and worldwide with 94-98% sequence identity and E-value zero after BLASTn were selected (Table 1) for phylogenetic analysis. Sequences were aligned using MUSCLE program in Mega7 and the completely aligned portion of 739bp was taken for study. Further comparison revealed a conserved region - (TYGARAAYGCYCARCCYACYTTTMGGCAAATYATG GCWCATTTTTCYGATGCYGSTGAGGMVTAYATAACA ATGCGYAAYRTYACRGARVGATRTATGCCYAGGTGG GGAGCRCTTASAGGATTGAMTGACATAARYYTAGCC CGWTATGCATTYGRTTTTWACGTAGKYACATCRAAA AMTACRARYAGGGCTAGAGARRYACAHACGCAGAT GRAAGMWGCAGCHATTCKTGG) from 359nt- 593nt in all the isolates with homozygosity of 0.977. Out of 616 sites analysed, 199 sites were variable.

Phylogenetic analysis

Phylogenetic tree deduced based on the nucleotide sequence of the CP gene (Fig. 1) showed a distinct divergence of BBrMV isolates in two major clades. Clade I included accession from USA, Thailand and Philippines while clade II included remaining. Isolate of Grand Naine (Kerala) was placed together in sub group with Cardamom (Kerala) with proximity of 0.018. It is far apart from isolate of USA having host plant Ginger (*Alpinia purpurata*) with a distance 0.053 (Table 1). It is at a distance of 0.036 with the remaining fifty-six isolates in the study. All isolates were placed at an average distance of 0.035 with each other.

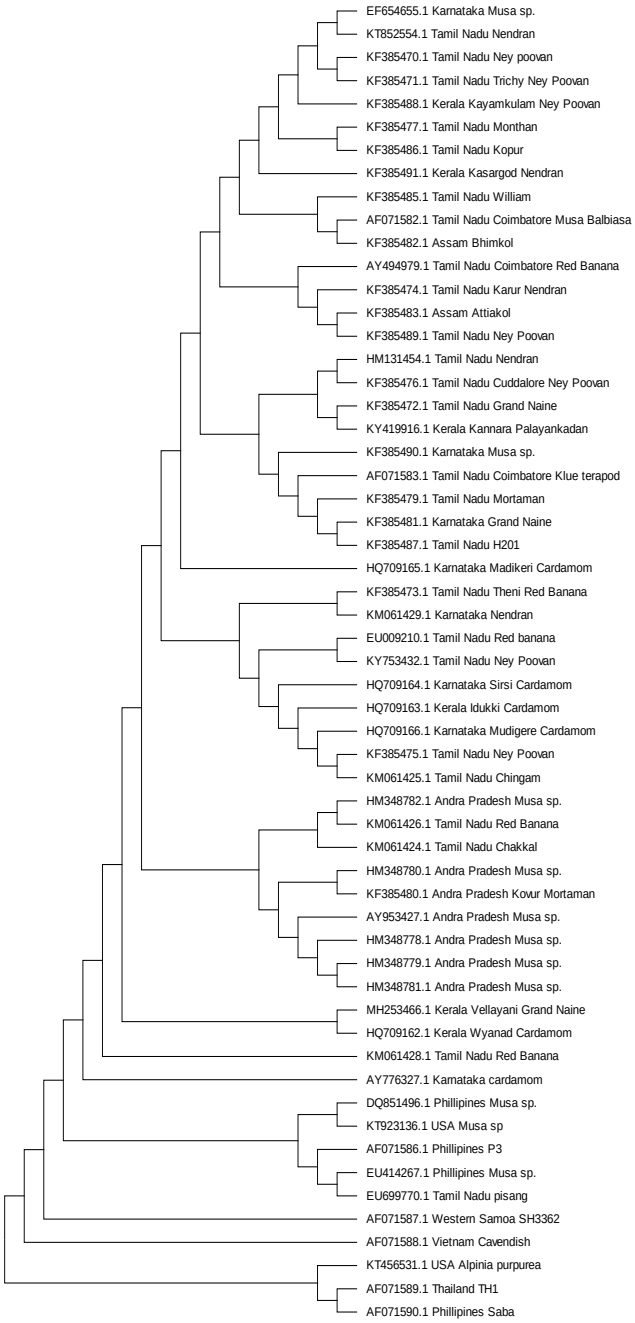

Fig 1: Phylogenetic analysis of coat protein of BBrMV isolates

Table 1: Accession number, host, region, query cover and sequence identity of BBrMV isolates with isolate from Grand Naine of Vellayani (Kerala)

S. No	Accession Number	Host	Place	Query cover	Identity	Phylogenetic distance
1.	AF071582	Musa Balbisian	Coimbatore	100%	97%	0.023
2.	AF071583	Klue Terapod	Coimbatore	100%	96%	0.035
3.	AF071586	P3	Phillipines (Davao)	100%	94%	0.057
4.	AF071587	Cv.SH3362	Western Samoa	98%	95%	0.053
5.	AF071588	Cavendish	Vietnam	100%	94%	0.051
6.	AF071589	Th1	Thailand	100%	94%	0.051
7.	AF071590	Saba	Phillipines (Losbanos)	100%	94%	0.062
8.	AY494979	Musa Sp	Coimbatore	99%	97%	0.027
9.	AY776327	Cardamom	Masegadde, Karnataka	100%	96%	0.032
10.	AY953427	Musa Sp	Andra Pradesh	99%	96%	0.032
11.	DQ851496	Musa Sp	Phillipines	100%	95%	0.046
12.	EF654655	Musa Sp	Karnataka	100%	96%	0.034
13.	EU009210	Red Banana	Trichy, Tamil Nadu	100%	97%	0.025
14.	EU414267	Musa Sp	Phillipines	100%	94%	0.053
15.	EU699770	Cv. Pisang	India	100%	94%	0.053
16.	HM131454	Nendran	Trichy, Tamil Nadu	100%	96%	0.034
17.	HM348778	Musa Sp	Andra Pradesh	99%	96%	0.037
18.	HM348779	Musa Sp	Andra Pradesh	99%	96%	0.030
19.	HM348780	Musa Sp	Andra Pradesh	100%	97%	0.030
20.	HM348781	Musa Sp	Andra Pradesh	99%	96%	0.037
21.	HM348782	Musa Sp	Andra Pradesh	99%	97%	0.030
22.	HQ709162	Cardamom	Wynad	99%	98%	0.018
23.	HQ709163	Cardamom	Idukki	100%	97%	0.028
24.	HQ709164	Cardamom	Sirsi	100%	98%	0.022
25.	HQ709165	Cardamom	Madikeri	100%	97%	0.027
26.	HQ709166	Cardamom	Mudigere	100%	97%	0.028
27.	KF385470	Ney Poovan	Tamil Nadu	100%	95%	0.048
28.	KF385471	Ney Poovan	Trichy, Tamil Nadu	100%	96%	0.037
29.	KF385472	Cv. Grande Naine	Theni, Tamil Nadu	100%	97%	0.028
30.	KF385473	Red Banana	Theni, Tamil Nadu	100%	98%	0.018
31.	KF385474	Nendran	Karur, Tamil Nadu	100%	97%	0.032
32.	KF385475	Ney Poovan	Tamil Nadu	100%	94%	0.059
33.	KF385476	Ney Poovan	Cuddalore, Tamil Nadu	100%	97%	0.034
34.	KF385477	Cv. Monthan	Tamil Nadu	100%	96%	0.044
35.	KF385479	Cv. Mortaman (AAB)	Trichy, Tamil Nadu	100%	96%	0.041
36.	KF385480	Cv. Mortman	Kovur, Andra Pradesh	100%	97%	0.032
37.	KF385481	Cv. Grande Naine	Banglore, Karnataka	100%	96%	0.035
38.	KF385482	Musa Balbisiana Cv. Bhimkol	Assam	100%	96%	0.037
39.	KF385483	Musa Balbisiana Cv. Attiakol	Assam	100%	96%	0.039
40.	KF385485	Cv. William (AAA)	Trichy, Tamil Nadu	100%	97%	0.022
41.	KF385486	Cv. Kapur (ABB)	Trichy, Tamil Nadu	100%	96%	0.046
42.	KF385487	Cv.H201(AB)	Trichy, Tamil Nadu	100%	96%	0.035
43.	KF385488	Ney Poovan	Kayamkulam, Kerala	100%	95%	0.039
44.	KY753432	Ney Poovan	Trichy, Tamil Nadu	100%	97%	0.027
45.	KF385489	Ney Poovan	Tamil Nadu	100%	95%	0.059
46.	KF385490	Musa Sp	Karnataka	100%	96%	0.036
47.	KF385491	Nendran	Kasargod, Kerala	100%	96%	0.034
48.	KM061424	Tamil Nadu local variety Chakkal	Thutukudi, Tamil Nadu	83%	97%	0.030
49.	KM061425	Tamil Nadu local variety Chingam	Kanyakumari, Tamil Nadu	83%	98%	0.023
50.	KM061426	Red Banana	Tamil Nadu	83%	98%	0.023
51.	KM061428	Red Banana	Tamil Nadu	83%	98%	0.018
52.	KM061429	Nendran	Karnataka	83%	97%	0.032
53.	KT456531	Alpinia Purpurea	USA	100%	94%	0.053
54.	KT852554	Nendran	Tamil Nadu	100%	96%	0.032
55.	KT923136	Agdia Control	USA	96%	95%	0.046
56.	KY419916	Cv. Palayankodan	Kannara, Thrissur	100%	97%	0.025
57.	MH253466	Cv. Grand Naine	Vellayani, Kerala	ref	ref	ref

Test of neutrality and Selection pressure analysis

Tajima's test of neutrality [21] compared the number of segregating sites per site with the nucleotide diversity and a value of -2.02856 was obtained in this study.

Secondary structure prediction

In secondary structure prediction, the percentage of beta sheet was found to be 68.2% greater than the percentage of helix (64.1%) and turn (11.5%). DAG motif was searched and identified in all the isolates in the study while, Rad4 motif was identified only in Grand Naine (Kerala).

Homology modelling of coat-protein and evaluation

A template sequence, 50DV, a crystal structure of a watermelon mosaic virus was the only protein which showed similarity with the CP query sequence of BBrMV. The template sequence was 59% identical with query sequence selected to predict the three-dimensional structure. CP structures were generated by Modeller 9v9, I-tasser and

Swiss-Model. The model predicted using Swiss-Model (Fig. 2a) was the best model based on structure validation scores (Table 2). The model structure obtained using Swiss-Model had 85.2% residues in the most favourable region of the Ramachandran plot (Fig. 2b) and showed ~93% ERRAT score. The Z-score obtained in protein structure analysis (Pro SA-web) was -3.78 and model quality was good.

Table 2: Structure validation scores using SAVES v5.0 server for coat protein of BBrMV

Test	Modeller 9v9	Swiss-Model	I-tasser Model 1	I-tasser Model 2	I-tasser Model 3	I-tasser Model 4
Verify	55.76%	65.14%	54.38%	66.82%	66.82%	61.50%
Errat	46.2687	93.16	43.4146	92.1569	92.118	89.2683
Prove	9.30%	0.00%	11.70%	9.30%	12.40%	10.20%
Procheck	87.60%	85.20%	82.50%	70.60%	68.60%	73.20%

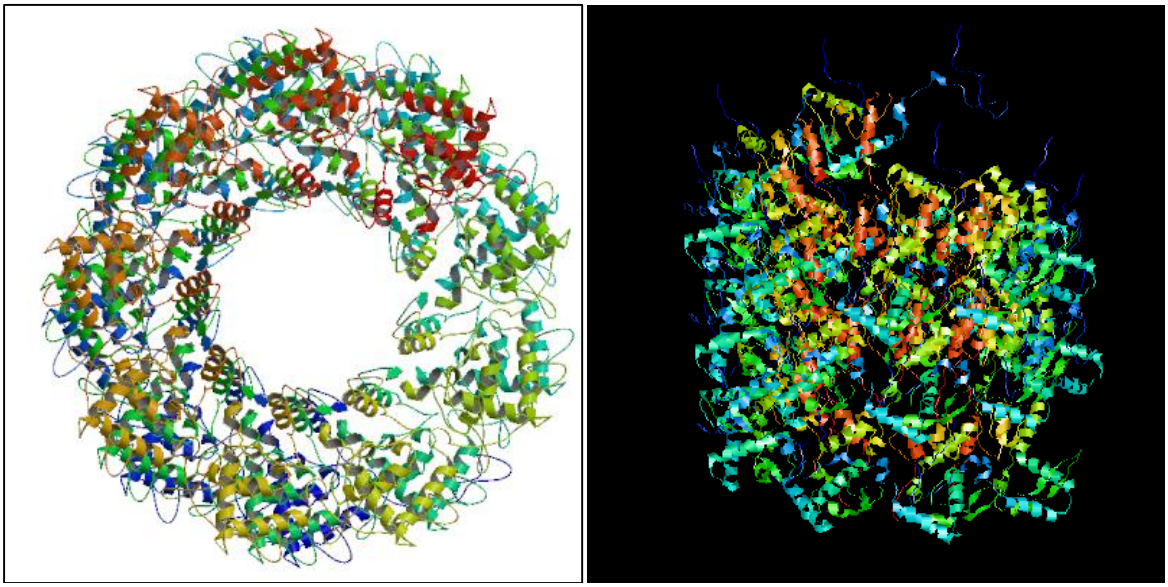

Fig 2a: Homology model of CP of BBrMV obtained by Swiss-Model

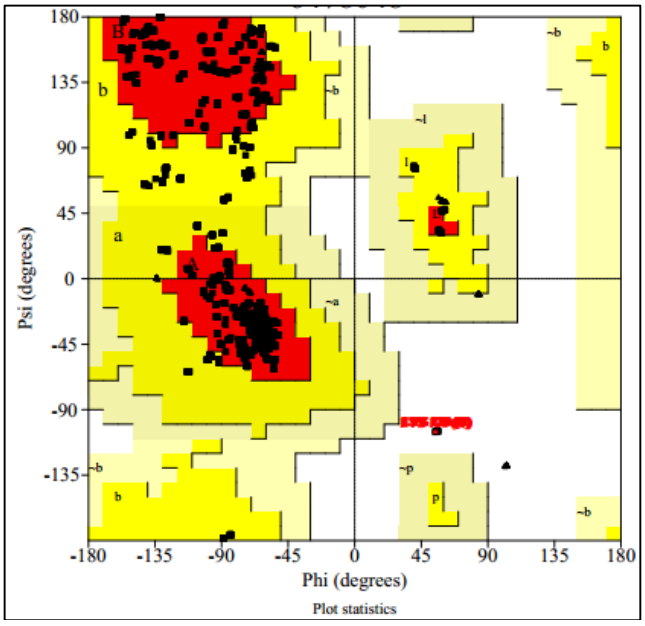

Fig 2b: Ramchandran plot for coat protein structure obtained by Swiss-Model

Discussion

In the present study CP sequence comparison with various isolates of BBrMV showed the presence of a conserved region of 234 nucleotides. The genetic diversity in BBrMV isolates in present study was found to be influenced by geographical area. A region wise grouping of isolates irrespective of the host was observed. *Pentalonia nigroneruosa* is common aphid found on cardamom, banana and flowering ginger which may have caused spread of BBrMV from banana host plant to flowering ginger [28] and cardamom [29]. Development of transgenic resistance against viruses is seen to be dependent on sequence homology among the isolates from different locations [30]. Based on sequence homology and evolutionary distance a broad-spectrum control strategy can be designed especially against RNA viruses which are prone to higher mutation rates.

RNA viruses possess an error-prone RNA dependent RNA polymerases (RdRp) which causes many errors during replication in RNA viruses. This is one of the reasons for purifying selection widely seen in RNA viruses [31]. Purifying selection helps virus to get rid of deleterious mutations. Based on Tajima's D value, purifying selection is also predicted in the present study in BBrMV isolates for the CP gene [32]. Purifying selection is supported by the secondary structure of proteins. The structures with more amino acid flexibility (turn and coils) accelerates the rate of evolution [33]. Hence, understanding the selection of secondary structures will help in investigating the occurrence of purifying selection and the sites that may be functionally critical in a region of a protein [34].

Sequence comparison showed the presence of DAG motif, a motif with crucial role in virus transmission through aphids. In all isolates DAG motif was present at N-terminal end in all isolates. The context in which DAG motif is present at N terminal is crucial for its role [35]. The studies on DAG motif in potyviruses suggested that the point mutations in the DAG motif [36] or the change in the amino acid preceding DAG motif [37, 38] can reduce the transmissibility. The probable reason may be reduction in the binding of CP with the Helper Component [36]. Another, Rad4 motif was identified only in

isolate from Grand Naine (Kerala). It is predicted to have function in recognition of lesions in the double helix and removal of damaged base pairs by inserting a b-hairpin through the DNA duplex [39]. Rad4 peptide is also reported to be a putative AC3 interactive peptide in ToLCKeV (Tomato Leaf Curl Kerala Virus) which may be involved DNA recombination and cell cycle pathway [40].

The 3D structure of CP obtained has a good stereo-chemical quality based on the percentage of residues in the core regions in Ramachandran plot and Z score. The structure of protein sets the foundation for its interaction with other molecules.

Conclusion

A conserved region was identified among the 57 CP isolates of BBrMV. Pattern of the nucleotide diversity gave the evidence of purifying selection with the influence of geographic location on variability of CP gene sequence. Protein structures with more turn and coils might be expected to have an effect on rate of evolution. DAG motif can be a putative target against viruses. Presence of rad4 motif in CP of Grand Naine isolate predicts the scope of recombination and evolution of CP. The CP structure can be used for drug designing. The information generated in this study can aid in establishment of broad-spectrum control strategies against BBrMV.

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