GENETIC DIVERSITY ANALYSIS, SEQUENCE MOTIF COMPARISON AND HOMOLOGY MODELING OF VPg FROM *BANANA BRACT MOSAIC VIRUS*

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INTRODUCTION

- *Banana bract mosaic virus* (BBrMV) belongs to the genus Potyvirus and is one of the most destructive viral diseases infecting bananas all over the world.
- VPg has been shown to take part in RNA replication, cell-to-cell and long-distance movement, translation, gene silencing suppression and phloem loading of the virus.
- VPg and its precursor forms play a central role in the viral replication cycle and the knowledge of VPg gene sequences and its structure will be useful in understanding the role of plant virus VPg in the initiation of RNA synthesis.
- Hence in this study we have compared the sequence diversity and the phylogenetic relationship, SNPs , INDELs, evolutionary distance and selection pressure analysis (Ka/Ks ratio) with known BBrMV isolates.

MATERIALS AND METHODS

- BBrMV-Try infected leaves were collected from Nendran cv. at Trichy
- Bioedit version 5.09.04 - Analysis of amino acid sequence data.
- Multiple sequence alignment - CLUSTAL W program.
- Phylogenetic analysis and evolutionary distance - MEGA 7.0 software.
- SNPs and INDELs - DnaSP version 5.10.
- Selection pressure - (http://www.datamonkey.org).
- PROSITE - Identification of motifs (http://prosite.expasy.org/).
- Homology comparative modeling (http://swissmodel. expasy.org/).
- PROCHECK (http://www.biochem.ulc.ac.uk/~roman/procheck/procheck.html/) and Swiss Model.

RESULT AND DISCUSSION

- The VPg gene was found to be 570 nucleotides (nt) coding for 190 amino acids (aa) and having a deduced MW of 21.54 kDa and pI of 9.03. Comparative sequence analysis of the BBrMV VPg isolates revealed 98% nt identity with BBrMV-Phi, BBrMV-Ind and 97% identity with BBrMV isolate infecting ginger and 97% AA sequence similarity with BBrMV-Phi and 96 % identity with BBrMV-Ind and BBrMV-Gin isolate, whereas it showed 53-98% nt and 39-97% aa identity with other potyviruses (Fig.1).
- BBrMV- Ind had an evolutionary distance of 0.016 from the reference sequence with 9 SNPs, the next closest sequences were of BBrMV-Phi with an evolutionary distance of 0.018 and 10 SNPs and BBrMV-Gin was farthest with an evolutionary distance of 0.027 and 14 SNPs. The values of Ka and Ks ranged from 0.006 to 0.01 and 0.01 to 0.015, respectively. The value of Ka/Ks ranged from 0.6-0.666. BBrMV-Gin showed high mutation at nucleotide level and protein level when compared with the reference isolate which may help in establishing the virus in two different hosts (Table 1). The data obtained from evolutionary distance, SNPs, INDELs and Ka/Ks ratio suggests the diversion of BBrMV-Gin from other banana isolates. The presence of no INDELs among BBrMV sequences and low Ka/Ks ratio suggests that though these sequences had the mutations, they may not alter the protein structure and hence function.
- Codon position 156 and 175 in VPg was detected under positive selection and mutations at this position significantly affect the self association of VPg as the region from 153 to 191 (38 aa) is important for the VPg self-interaction.
- A number of conserved and divergent functional motifs were identified in this study which may be essential for performing multiple functions, thereby emphasizing fundamental and species-specific mechanisms (Table 2). A computational approach following homology modeling protocol has been used to predict the 3D structure of VPg (FIG. 2).

Table 1: Analysis of evolutionay distance, SNP, Indels and Ks/Ka ratio and percentage sequence identity of VPg gene of BBrMV isolate

S.No	Isolate	Accession No.	Evolutionary Distance	SNP	Ka	Ks	Ka/Ks	NT Identity %	AA Identity %
1	This Study	KT852552	-	-	-	-	-	-	-
2	BBrMV-Phi	YP001718528	0.018	10	0.0007	0.011	0.636	98	97
3	BBrMV-Ind	AEC46819	0.016	9	0.006	0.010	0.600	98	96
4	BBrMV-Gin	ANA04446	0.027	14	0.010	0.015	0.666	97	96

Fig. 1 Multiple sequence alignment of VPg from different potyviruses including BBrMV isolates

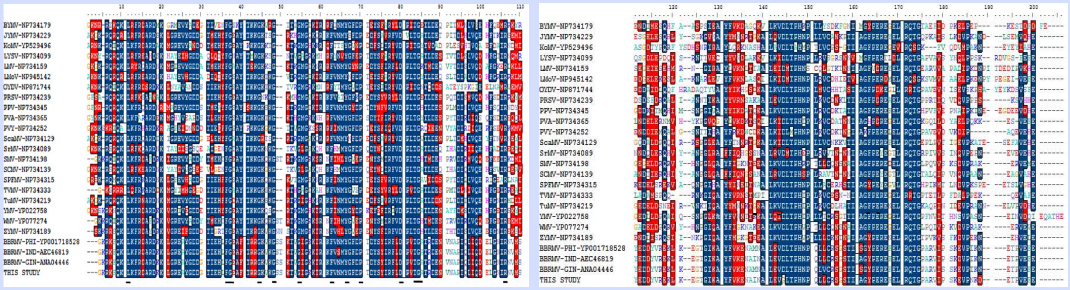
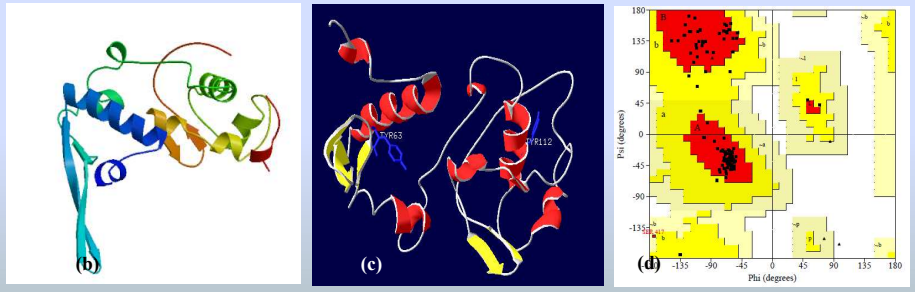


Table 2: The sequence motifs identified in VPg of *Banana Bract Mosaic Virus*

Motif sequence	Location	Domain
KL	7-8	N-Terminal
AY	22-23	N-Terminal
GDD	27-29	N-Terminal
FG	33-34	N-Terminal
AF	36-37	N-Terminal
TRRGRVK	38-44	N-Terminal
GRVKGSSKTVG	43-51	N-Terminal
F	59	N-Terminal
YG	63-64	N-Terminal
P	67	N-Terminal
DP	77-78	N-Terminal
TG	80-81	N-Terminal
Q	94	N-Terminal
R	103	Central Domain
AY	125-126	Central Domain
L	136	Central Domain
DL	139-140	Central Domain
PH	142-143	Central Domain
I	155	C-Terminal
YP	158-159	C-Terminal
E	162	C-Terminal
LR	165-166	C-Terminal
G	169	C-Terminal
P	180	C-Terminal

Fig. 2 3D Structure of BBrMV HC-Pro. (a) Diagram of the BBrMV RNA genome, the domain organization of VPg, and the region used for modelling. (b) 3D structural model of BBrMV VPg. (c) 3D model of BBrMV VPg showing the Tyr^{63} and Tyr^{112} (blue) are shown as *sticks*. (d) Ramachandran plot for the BBrMV VPg



CONCLUSION

- High sequence similarity at nt and aa levels suggested that BBrMV VPg from banana do not vary considerably and represent similar architecture except for the isolate from ginger.
- VPg genes are subjected to strong purifying selection which may play an important role in the host virus interaction and in shaping evolution. Knowledge of BBrMV genetic diversity based on VPg is crucial to the development of efficient and stable control strategies.
- In conclusion, this study provides information on various sequence motifs, domains of BBrMV VPg to relate its biological functions in understanding its role in plant-virus interaction.