Journal of Scientific Research and Reports

Volume 30, Issue 4, Page 240-251, 2024; Article no.JSRR.113532
ISSN: 2320-0227

In silico Characterization of Banana Bunchy Top Virus (BBTV) and Its Genetic Variation Globally

Rishee K. Kalaria [a*] and R. M. Patel [a]

[a] *Aspee Shakilam Biotechnology Institute, Navsari Agricultural University, Surat, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2024/v30i41910

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/113532

Original Research Article

Received: 01/01/2024
Accepted: 03/03/2024
Published: 06/03/2024

ABSTRACT

Banana bunchy top disease is one of the most prevalent viral infections associated with banana farming. It is a rapidly spreading disease. Currently, there are very few fully sequenced isolate reports from India and around the world. When in silico analysis was conducted on various Banana Bunchy Top Virus (BBTV) various genome components at nucleotide level (DNA-R and DNA-U3), and amino acid level (DNA-C and DNA-U3) maximum genetic variability was observed in all reported strains of BBTV. When comparing DNA-R (Replicative protein) to DNA-S segment (Coat protein) most Indian isolates are in agreement with isolates from countries in East and Southeast Africa and belong to the PIO (Pacific-Indian Oceans) group of BBTV isolate classification. BBTV coat protein model demonstrated the highest degree of protein binding to NBS-LRR class resistance protein. Furthermore, the Ramachandran plot was used to validate the BBTV CP model, and Procheck and the PROSA web server were used to enhance the structure. The greatest binding affinity of the BBTV coat protein was seen with the NBS-LRR class resistance protein (receptor) when the BBTV coat protein model interacted with it. Three mutagenic epitope (IADEFYVERL, SKRFLLVLDD and WEFFKQCAFSS) were predicted from BBTV coat protein

**Corresponding author: E-mail: risheekal@nau.in;*

region. Consequently, our work presents the Indian subcontinent as a possible hotspot for rapid demographic development from an extremely small viral population size, adding significantly to the knowledge already known on BBTV worldwide.

Keywords: Banana; BBTV; Epitope; Bunchy top virus; Begomovirus; Ramachandran plot and Mutagenecity.

1. INTRODUCTION

The banana (*Musa paradisica* Mill.), a fruit crop that is widely cultivated and has a high production value and economic benefits while requiring little maintenance, is a member of the *Musaceae* family. Owing to its exceptional nutritional value, it constitutes one of the most important horticultural food crops planted in tropical and subtropical countries [1-3]. The Banana Bunchy Top Virus (BBTV), which is classified as one of the most devastating viruses on the top ranking of "World's worst invasive species list," belongs to the Genus *Babuvirus* and family *Nanoviridae* [4], causing limited production. The terrible viral disease that affects bananas is called banana bunchy top disease (BBTV). Since its discovery in 1889 in Fij, BBTV has catastrophically destroyed numerous banana varieties in all of the worldwide significant banana-growing locations over the past several years [3,5]. BBTV is transmitted in persistent, circulative manner by the black banana aphid (*Pentalonia nigronervosa*) [6,7]. The term "bunchy top" originates from the rosette-like structure of the diseased plants that exhibit severe signs, which have thin, erect, and gradually smaller leaflets. Often, the margins of the leaves curl upward and exhibit a slight browning. Midrib and petiole frequently have dark green stripes that run down into the pseudo stem. The multi-component, circular, DNA with one strand virus known as BBTV is a member of the family *Nanoviridae* and genus *Babuvirus*. Numerous molecular analyses of the BBTV genome have demonstrated that the virus possesses isometric virions [3,8], The isometric virion measures 18–20 nm in diameter. It is transmitted by banana black aphid (*P. nigronervosa*) in a persistent manner. The BBTV genome is made up of at least six essential parts, each measuring around 1 KB (BBTV DNA-R, -S, -M, -C, -N and -U) BBTV DNA-R encodes a master replication initiation protein (Rep), DNA-S encodes a viral coat protein (CP) for encapsulation, DNA-M encodes for movement protein, DNA-C encodes for cell cycle link protein, DNA-N encodes for nuclear shuttle protein while DNA-U encodes unknown function [9].

The Banana bunchy top disease has spread extensively over the globe, mostly to the continents of Africa, Australia, and Asia Pacific, as well as to other South Pacific Islands, however reports of it in American countries have not yet been received [10,11,12]. This has been made feasible by an increase in human-mediated travel, the global trade in agriculture in bananas, and inadequate sanitary laws [13]. Gujarat has the greatest production in India among the several states, averaging 177.5 metric tonnes compared to the national average of 35.50 metric tons. Numerous variables that limit productivity, including pests and illnesses, have an impact on bananas. Diseases such as banana bunchy top virus (BBTV), wilt (*Fusarium oxysporum*), and black sigatoka (*Mycosphaerella fijiensis*) result in large reductions in yield [14-16].

Despite being a large producer of bananas, only a small number of instances of BBTV infection have been thoroughly investigated in several of India's districts [17-21]. Lack genetic variation, sterility of most edible cultivars, long reproduction times, and varying ploidy levels have hindered the development of disease-resistant Musa through traditional breeding methods [22,23]. To control bunchy top disease in bananas, a number of methods have been tried, but none of them have been able to provide complete protection. Compared to many other severe viral diseases, research into the fundamental reason of resistance to this disease has progressed extremely slowly because of the challenges associated with purifying the virus [24-26]. Therefore, the present study was under taken on *in silico* characterization of banana bunchy top virus (BBTV). Our primary goal was to investigate attempts to genetically distinguish BBTV isolates. By comprehending the evolutionary tendency, we will undoubtedly be able to better disease management in the future and support protected agriculture practices worldwide.

2. MATERIALS AND METHODS

2.1 Identified Amino Acid and Nucleotide Variation among Each Genome Components of BBTV

Different segment or component wise sequence retrieved from NCBI database to find the variation in different nucleotide sequences for conserved sequence and motif using motif scan tool(https://www.genome.jp/tools/motif/) and MEGA X v.10.0.7[27,28]. Similarly different segment wise protein sequence were retrieved from NCBI database to find conserved domain using NCBI CCD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) available respectively. Later analyzed to determine the optimal nucleotide and amino acid substitution model and generate a Neighbor joining phylogenetic evolutionary tree (1000 bootstrap replicates) using MEGA X v10.0.7.[27,28].

2.2 Structure Prediction of BBTV Coat Protein

Complete BBTV Coat-protein sequence was also used in a BLASTP-based homolog search with RCSBPDB (PDB; http://www.rcsb.org/pdb/home/home.do) to determine homologs. Following BLASTP, template sequences in fasta format with at least 30% sequence identity were obtained from RCSB-PDB. Afterwards, we decide to use the online I Tasser web server for abinitio modeling of coat protein (https://zhanggroup.org/I-TASSER/). The model's built-model quality was assessed by analyzing the amino acid area in the Ramachandran plot on the Pro Check web server.(https://servicesn.mbi.ucla.edu/PROCHECK/) [29,30,31]. The models were chosen for additional investigation based on the frequency of outliers and favoritism percentage. Later Refinement of model was done by PROSA web server(https://prosa.services.came.sbg.ac.at/prosa.php).

2.3 Prediction of Antigenic Mutagenecity Peptide from BBTV Coat Protein

BBTV coat protein model was tested as interaction with *Musa acuminata* NBS-LRR class resistance protein using GalaxyPepDock web server(https://ardock.ibcp.fr/). Later, conserve epitope sequences from BBTV coat protein predicted using different web tool ie SVMTriP(http://sysbio.unl.edu/SVMTriP/prediction.php), NOVAPROLABS (https://www.novoprolabs.com/tools/peptide-antigen design) , PREDICTED ANTIGENIC PEPTIDES (http://imed.med.ucm.es/ Tools/antigenic.pl) Emboss(http://www.bioinformatics.nl/emboss) available for antigenic mutagenecity from available amino acid sequences.

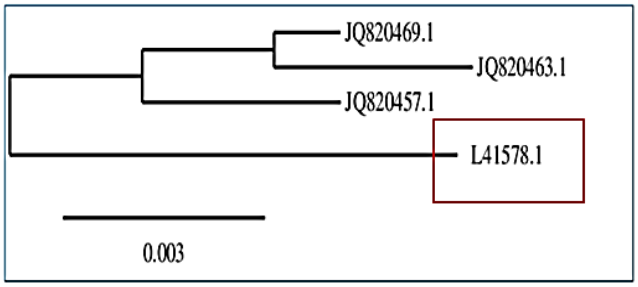
3. RESULTS AND DISCUSSION

Total six segments ie DNA C,DNA M,DNA N, DNA R,DNA S and DNA U3 respectively different nucleotides sequences retrieved from NCBI taking Bio Project: PRJNA485481 as reference . Segment DNA C which mainly encodes for cell cycle link protein in BBTV genome, where L41578.1 (reference), JQ820457.1, JQ820463.1, JQ820469.1, Total 13 conserved sequence and total 32 conserved motif were found using motif scan tool. Phylogenic analysis revealed L41578.1 different cluster from JQ820457.1, JQ820463.1, JQ820469.1 respectively. Segment DNA –M encodes for movement protein in BBTV genome, where L41575.1 (reference), JQ820456.1, JQ820462.1, JQ820468.1, MG545613.1 , MG545614.1 (Fig.1, Fig. 7, Fig. 8, Fig. 9, Fig. 10, Fig. 11, Fig. 12).

Total 2 conserved sequence and 54 motif were found using motif scan tool. Phylogenic analysis revealed L41575.1 found in different cluster compare to JQ820456.1, JQ820462.1, JQ820468.1 respectively. Segment DNA N encodes for nuclear shuttle protein in BBTV genome, where L41577.1 (reference), EF529519.1, JQ820458.1, JQ820464.1, JQ820470.1 , MG545615.1. Total 2 conserved sequences and 61 motif were found using motif scan tool. Phylogenic analysis revealed L41577.1 found in different cluster compare to EF529519.1, JQ820458.1, JQ820464.1, JQ820470.1 respectively. Segment DNA R encodes for Replicase protein in BBTV genome, where S56276.1 (reference), JQ820453.1, JQ820459.1, JQ820465.1, MG545610.1, MG545615.1, MG545614.1, MG545613.1, MG545612.1. Total 43 motif were found using motif scan tool. Phylogenic analysis revealed S56276.1 found in same cluster with JQ820459.1 and JQ820465.1. Segment DNA S encodes for Coat protein in BBTV genome, where L41574.1 (reference), JQ820455.1, JQ820461.1, JQ820467.1, MG545612.1. Total 6 conserved sequence and 49 motif found using motif scan tool. Phylogenic analysis revealed L41574.1 found in same cluster with

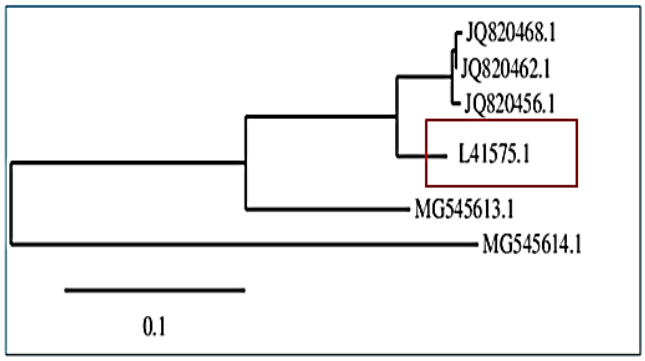
JQ820455.1, JQ820461.1, JQ820467.1.Segment DNA U3: encodes unknown function in BBTV genome, where L41576.1 (reference), GQ214699.1, FJ773283.1, EU366170.1, FJ859750.1, FJ859749.1, JQ820454.1, JQ820460.1, JQ820466.1, FJ859748.1. In DNA-R (Replicase protein) majority of Indian isolates matches with isolates of region Rwanda(Country in East Africa) and Malawi(Southeastern Africa) compare to rest majority isolates from china whiles DNA-S segments (coat protein) majority of indian isolates matches with isolates of region Malawi(Southeastern Africa), Rwanda(Country in East Africa) and DR Congo(Central Africa) compare to rest isolates from DR Congo(Central Africa) . Total 61 motif and no conserved region found using motif scan tool. Phylogenic analysis revealed L41576.1 found in different cluster compare to all (Fig.. 2, Fig. 13, Fig.. 14, Fig. 15, Fig.. 16, Fig. 17, Fig. 18).

Phylogeny analysis of all DNA–C segments region (Nucleotide)



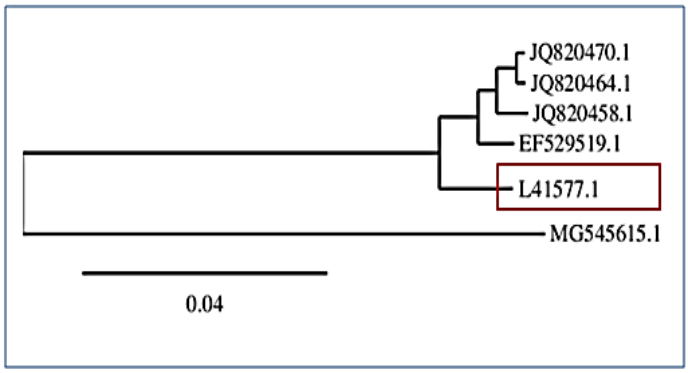
Bootstrap value: 1000 and Phylogenic method: Neighbor-joining

Phylogeny analysis of all DNA–M segments region



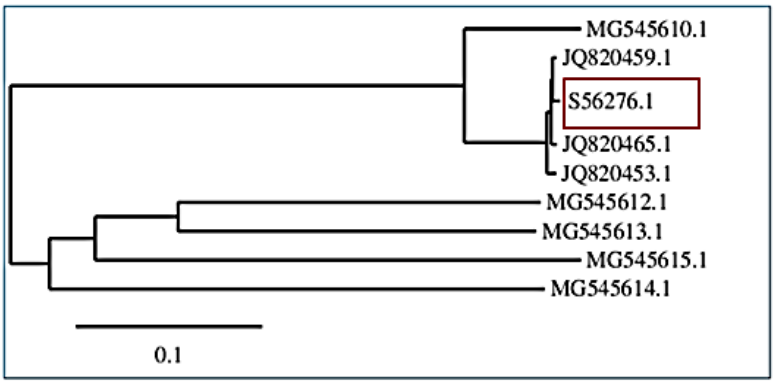
Bootstrap value: 1000 and Phylogenic method: Neighbor-joining

Phylogeny analysis of all DNA–N segments region



Bootstrap value: 1000 and Phylogenic method: Neighbor-joining

Phylogeny analysis of all DNA–R segments region



Bootstrap value: 1000 and Phylogenic method: Neighbor-joining

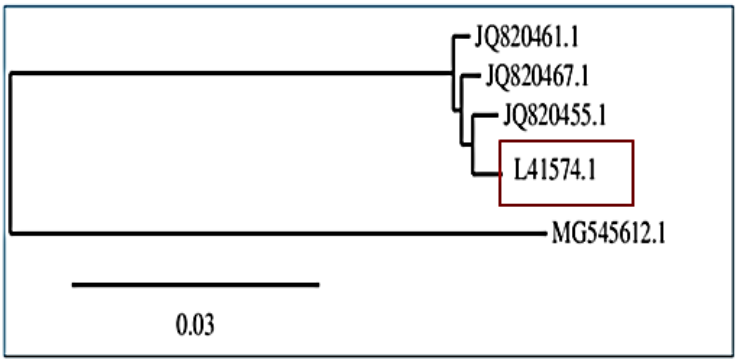
NCBI database : **S56276.1 (as reference)** , JQ820453.1 , JQ820459.1 , JQ820465.1 , MG545610.1 , MG545615.1 , MG545614.1 , MG545613.1 , MG545612.1, **AY845437.1, AF102780.1, FJ605506.1, HM120718.1, KU559329.1,KX868958.1, U18077.1, AY996562.2, HQ259074.1(Indian sequences)**

Note: Indian sequence were partial cds

Phylogeny analysis of all DNA–R segments region

Bootstrap value: 1000 and Phylogenic method: Neighbor-joining

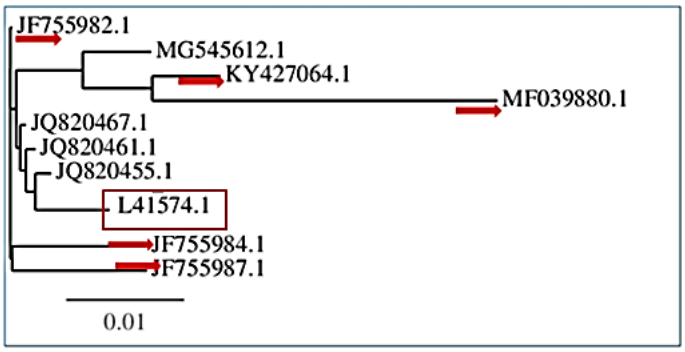
Phylogeny analysis of all DNA–S segments region



Bootstrap value: 1000 and Phylogenic method: Neighbor-joining

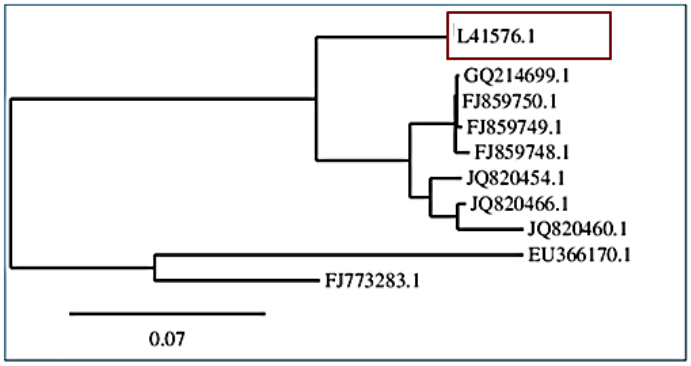
NCBI database : L41574.1 **(as reference)** , JQ820455.1 , JQ820461.1 , JQ820467.1 , MG545612.1, **MF039880.1, KY427064.1, JF755984.1,JF755982.1, JF755987.1 (Indian sequences)**

Note: Indian sequence were partial cds **Phylogeny analysis of all DNA–S segments region**



Bootstrap value: 1000 and Phylogenic method: Neighbor-joining

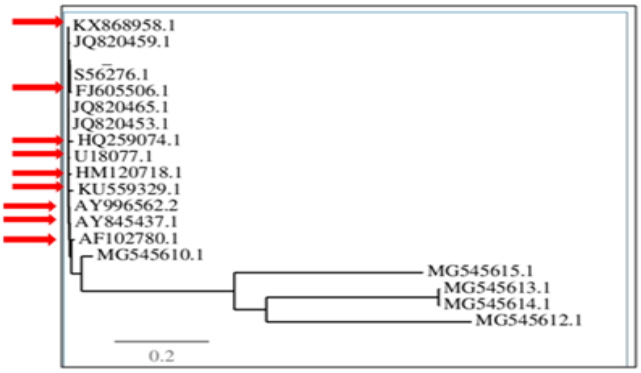
Phylogeny analysis of all DNA–U3 segments region



Bootstrap value: 1000 and Phylogenic method: Neighbor-joining

Fig. 1. Nucleotide variation of different genome components of BBTV

Phylogeny analysis of all DNA–R segments region (Protein)



On base of percent identity in nucleotide sequence and protein sequence of Replicase protein

Bootstrap value: 1000 and Phylogenic method: Neighbor-joining

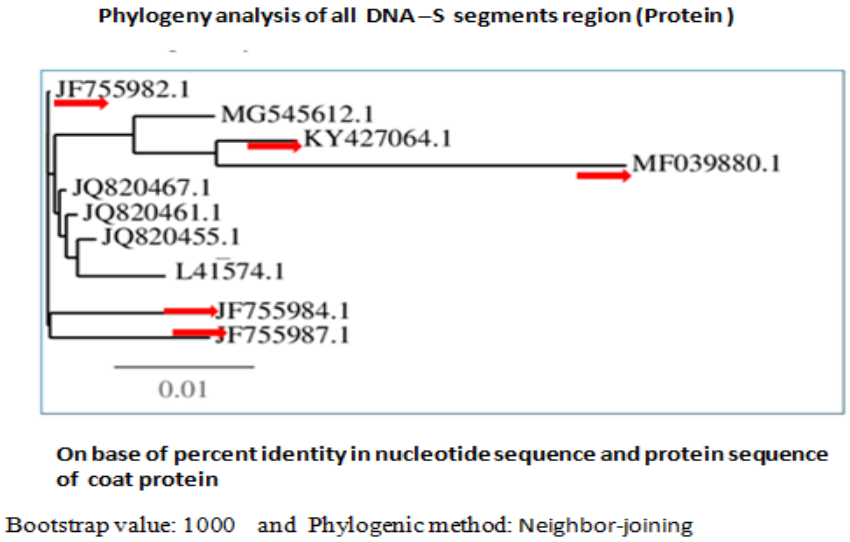


Fig. 2. Amino acid variation of replicase and coat protein components of BBTV

Similar work was carried out [31] to find HIV genome wide variation. Similar research was carried out by [32] to find nucleotide variations in human genome Similarly Belmabrouk and his coworker [33] carried out nucleotide variation in different BBTV genome component. Conserved Domain was present in segment DNA M as Babuvirus MP super family,DNA U3 as Nanovirus C8 super family, DNA R as Viral Rep and P loop NTPase super family, DNA S as nanovirus coat super family. Homology of BBTV coat protein was carried out by search with pdb database for similarity was 36% (>50) then decide to go for abinitio modeling. Predicted BBTV CP model validation with Ramachandran plot using Rampage revealed that 83.3% aa in favoured region. Further Prosa (refinement and validation of protein structures prediction and modelling based on energy criteria validation) result revealed that 89.1% aa in favoured region. Similar research was carried out by Prajapati and his coworker [34] predicted 3D structure of AC1 Proteins of Begomovirus. Similarly Patel and kalaria [29] carried out papaya ring spot virus coat protein prediction.

Later Identification of coat protein binding receptor in banana genome or any other plant genome and its interaction with BBTV coat protein revealed HQ704837.1 as result of BLASTP. Due to less similarity in PDB database later sequence was taken for 3D structure prediction using I Tasser web server (Fig. 3). Similarly Ravindra and Kalaria [31] carried out TLCV coat protein prediction using homology modelling.

Vigna mungo disease resistance protein CYR1 (CYR1) mRNA, complete cds (aa) then Check above sequence similarity with (Musa (taxid:4640)) in NCBI then show 79.55% sequence similarity with Musa acuminata AAA Group NBS-LRR class resistance protein (BR-4) mRNA, partial cds (EF515836.1). An essential phase in the structural modeling process is evaluating the model's performance. After the model is constructed, it needs to be examined using the checking tools provided to determine whether the stereochemistry of the model is relatively dependable and that the usual values come from crystal structures. The accuracy of the simulated structure provided by I Tasser web server model computations was assessed using a Ramachandran plot calculation in the PROCHECK tool validation package. The result revealed 83.3% amino acid in most favourable region with 130 amino acid. Later the model was configure in PROSA web server result in 89.1% amino acid in most favourable region with 171 amino acid (Fig. 4). The GalaxyPepDock web service was utilized to test the BBTV coat protein model in order to identify any interactions with the *Musa acuminata* NBS-LRR class resistance protein engaging in molecular docking with both interacting residues (Fig. 5).

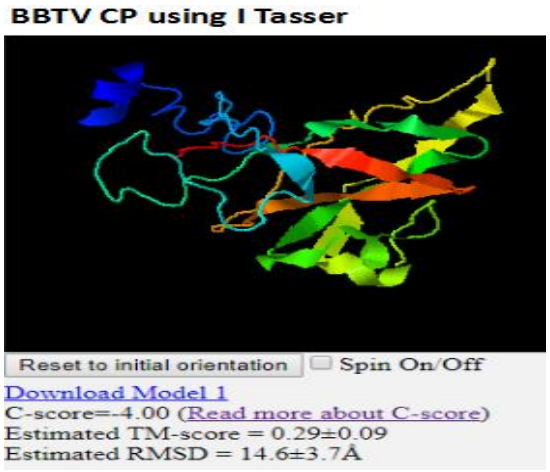
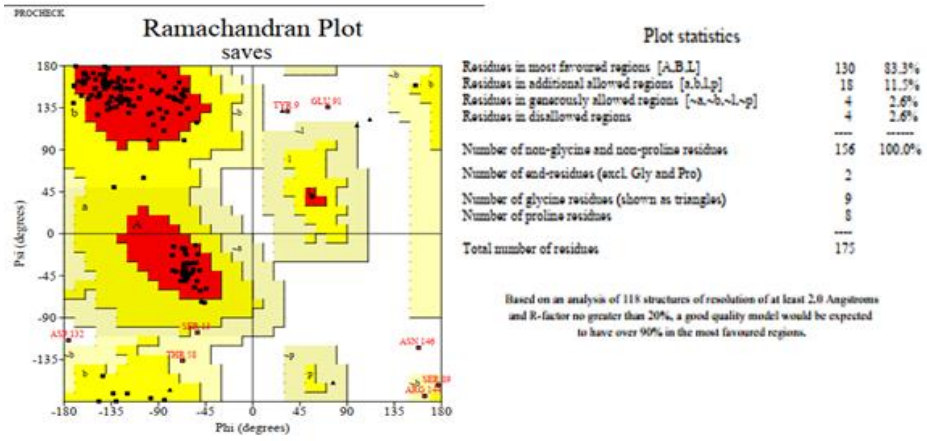


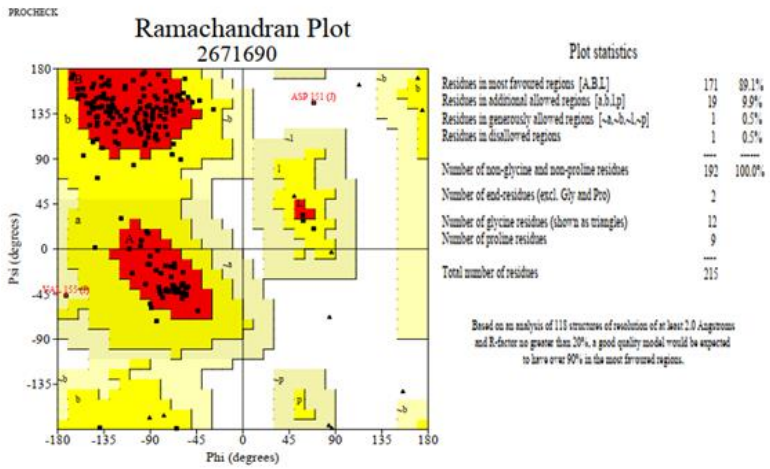
Fig. 3. Prediction of BBTV coat protein structure using I Tasser Server

Predicted BBTV CP model validation with Ramachandran plot (I Tasser model)



Model predicted using **I Tasser show 83.3%% aa** in favoured region

Predicted BBTV CP model validation with Ramachandran plot after refinement tool PROSA



Model predicted using **I Tasser show 89.1% aa** in favoured region

Fig. 4. Predicted BBTV CP model validation with ramachandran plot

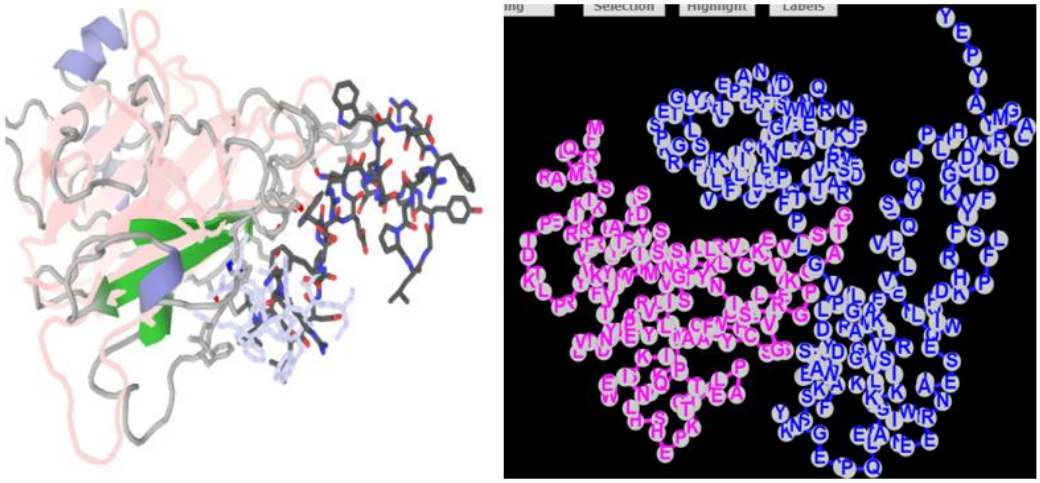


Fig. 5. Predicted BBTV coat protein model interaction with *Musa acuminata* NBS-LRR class resistance protein

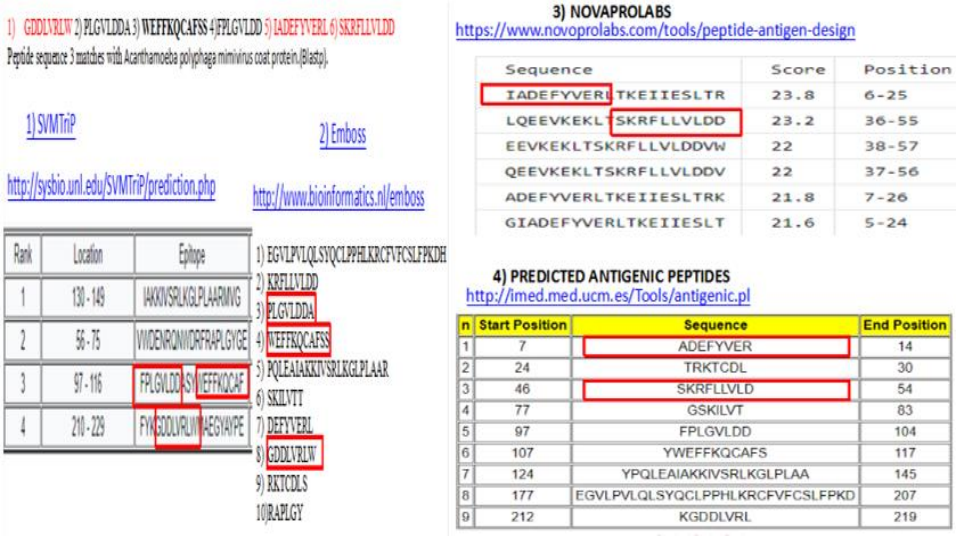


Fig. 6. Prediction of antigenic mutagenecity peptide for BBTV coat protein

Similar research was carried out by meena and his co worker [35] to predicted *in silico* homology of Garlic Common Latent Virus Coat-protein. Similarly Ravindra and Kalaria [31] carried out TLCV coat protein model refined using PROCHECK tool where number of amino acid were increased in favourable region. Similarly, Likhith and Peter also carried out *in silico* analysis of Coat Protein (CP) of Tomato Leaf Curl Virus (ToLCV) and Tomato Yellow Leaf Curl Virus (TYLCV). Later these models interacted with GroEL Protein of *Bemisia tabaci* to find out the interactive amino acid respectively[36].

Later NBS-LRR class resistance protein of *Musa acuminata* search for conserved amino acid residues with pfam database 1) KRFLLVLDDVWDENRQNWDRFRAPLGYGEP 2) VLPVLQLSYQCLPPHLKRCFVFCSLFPKD. Later prediction of antigenic mutagenecity peptide for BBTV coat protein result revealed 1)GDDLVRLW 2) PLGVLDDA 3) WEFFKQCAFSS 4)FPLGVLDD 5) IADEFYVERL 6) SKRFLLVLDD(Fig. 6).

Later three peptides sequence ie (1) GDDLVRLW, 2) IADEFYVERL,3) SKRFLLVLDD found as interacting amino acids of BBTV coat protein with NBS-LRR class resistance protein. Tian and his coworker [37] predicted mutagenic peptide using Potato virus Y Coat Protein Epitopes for Commercial monoclonal antibodies. This expands our knowledge of the mechanisms

involved in antibody binding and helps create epitope-based diagnostic instruments, disease resistance, and management plans for bananas against BBTV, an economically significant crop species that causes significant production loss.

4. CONCLUSION

To conclude, our research findings for the first time has reported at nucleotide level of BBTV genome component, DNA –C showed maximum sequence similarity with 13 conserved region with 32 conserved motif while DNA-M and DNA-N both showed least conserved region 2 with 54 and 61 conserved motif respectively while no conserved region found in DNA-R and DNA-U3 among the different strains. At amino acid level of BBTV genome component, DNA –R showed 2 conserved domain while DNA –M , DNA –N , DNA –S each showed 1 conserved domain respectively while DNA-C and DNA-U3 showed no conserved domain among the different strains.In DNA- R (Replicase protein) majority of Indian isolates matches with isolates of region Rwanda(Country in East Africa) and Malawi(Southeastern Africa) compare to rest majority isolates from china whiles DNA-S segments (coat protein) majority of indian isolates matches with isolates of region Malawi(Southeastern Africa), Rwanda(Country in East Africa) and DR Congo(Central Africa) compare to rest isolates from DR Congo(Central Africa). Further the BBTV CP model was validated using Ramachandran plot and refinement of structure was done using Procheck and PROSA web server. BBTV coat protein model were interacted with NBS-LRR class resistance protein where maximum binding affinity of BBTV coat protein was observed with NBS-LRR class resistance protein (receptor) was predicted. Total 6 mutagenic epitope 1) GDDLVRLW 2) PLGVLDDA 3) WEFFKQCAFSS 4) FPLGVLDD 5) IADEFYVERI 6) SKRFLLVLDD of BBTV CP matches with *Acanthamoeba polyphaga mimivirus* coat protein out of which 3 mutagenic epitope (1) GDDLVRLW, 2) IADEFYVERL,3) SKRFLLVLDD found as interacting amino acids with NBS-LRR class resistance protein. Generally more than 10 aa peptide are considered as epitope peptide. Furthermore, since the BBTV population is expanding globally, this study will motivate researchers to carry out more studies in other uncharted areas where banana farming is common. It will also be helpful in laying the groundwork for a more advanced and targeted detection mechanism for upcoming diagnostic tools.

APPENDIX

Appendix is available in the following link:
https://journaljsrr.com/media/Appendix_2024_JSRR_113532.pdf

ACKNOWLEDGEMENTS

Success is the result of hard work, dedication, inspiration, motivation, and creativity. It is my proud privilege to express my heartfelt appreciation to Dr R. M. Patel, ASBI, NAU, Surat for his generous assistance, unwavering guidance, supervision, critical suggestions, and positive attitude towards my abilities, which enabled me to complete this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chakraborty S, Dutta S, Barman M ,Samanta S, Sarkar K P, Poorvasandhya, R, Tarafdar J. Detection and *in silico* characterization of banana bunchy top virus in West Bengal, India: relevance to global genetic diversity and population structure. Virus Disease. 2023;34:221–235
2. Anonymous. retrieved from FAO; 2020. Available:https://www.fao.org/faostat/en/#data/QCL.
   Accessed 25 April 2023.
3. Chandrasekar A, Kalaiponmani K, Angappan K. Banana bunchy top viral coat protein (CP) gene expression studies at molecular level in hill banana cv. Sirumalai (AAB). International Journal of Current Microbiology and Applied Sciences. 2017; 6(6):398-411.
4. IPPC (International Plant Protection Convention). Banana bunchy top virus; 2010.
   Accessed 12 Oct 2023
   Available:http://www.ippc.int/file_uploaded/1162364592814_List_of_Regulated_pests.doc
5. Jones DR. Introduction to banana abaca and enset. In: Jones DR, editor. Diseases

of banana abaca and enset. CABI Publishing Wallingford. 2000;1–27.
6. Singh RP, Kurz J, Boiteau G. Detection of stylet-borne and circulative potato viruses in aphids by duplex reverse transcription polymerase chain reaction. The Journal of Virological Methods. 1996;59:189-196
7. Valerie K, Thomas JE, Sharman M, Mademba-Sy F. First record of banana bunchy top disease in New Caledonia. Australasian Plant Pathology. 2001;30:71
8. Su HJ, Tsao LY, Wu ML, Hung TH. Biological and molecular categorization of strains of BBTV. The Journal of Phytopathology. 2000;151:290-296
9. Thomas, JE, Dietzgen, RG. Purification, characterization and serological detection of virus-like particles associated with banana bunchy top disease in Australia. Journal of General Virology. 1991;72:217-224
10. Amin I, Qazi J, Mansoor S, Ilyas M, Briddon, RW. Molecular characterization of banana bunchy top virus from Pakistan. Virus Genes. 2008;36:191–8.
11. Diekmann M, Putter CAJ. editors. Musa spp. Bioversity International. Rome: FAO/IPGRI. 1996;28
12. Chakraborty S, Barman M, Samanta S, Roy M, Tarafdar J. Effect of banana bunchy top virus on the heat shock protein genes of *Pentalonia nigronervosa* during temperature susceptibility and its effect on virus transmission. Agronomy Journal. 2021;11(9):1866.
13. Stainton D, Martin DP, Muhire BM, Lolohea S, Halafihi MI, Lepoint P, Blomme G, Crew KS, Sharman M, Kraberger S, Dayaram A. The global distribution of Banana bunchy top virus reveals little evidence for frequent recent human-mediated long distance dispersal events. Virus Evolution. 2015;1(1):1.
14. Dale JL. Banana bunchy top: An economically important tropical plant virus disease. Advances in Virus Research.1987;33:301- 325
15. Tripathi, L, Tripathi, JN, Tushemereirwe, WK. Strategies for resistance to bacterial wilt disease of bananas through genetic engineering. African Journal of. Biotechnology: 3(12):688-692
16. Chandrasekar A, Kalaiponmani K, Elayabalan S, Kumar KK, Angappan K, Balasubramanian P. Screening of banana bunchy top virus through multiplex PCR approach. Archives of Phytopathology and Plant Protection. 2001;19:1920- 1925.
17. Banerjee A, Roy S, Behere GT, Roy SS, Dutta SK, Ngachan SV. Identification and characterization of a distinct banana bunchy top virus isolate of Pacific-Indian oceans group from North-East India. Virus Research. 2014;183:41–9.
18. Das T, Banerjee A. Distribution molecular characterization and diversity of banana bunchy top virus in Tripura India. Virus Diseases. 2018;29(2):157–66.
19. Islam MN, Naqvi AR, Jan AT, Haq QMR. Genetic diversity and possible evidence of recombination among banana bunchy top virus (BBTV) isolates. International Research Journal of Microbiology. 2010;1(1):1–12.
20. Kavino M, Harish S, Kumar N, Saravanakumar D, Samiyappan R. Molecular characterization of coat protein and nuclear shuttle protein genes of banana bunchy top virus from Western Ghats in India. Archives of phytopathology and plant protection. 2011;44(5):405–11.
21. Selvarajan R, Sheeba M, Balasubramanian V, Rajmohan R, Dhevi NL, Sasireka T. Molecular characterization of geographically different banana bunchy top virus isolates in India. Indian Journal of Virology. 2010;21(2):110–6.
22. Karan M, Harding RM, Dale JL. Evidence for two groups of banana bunchy top virus isolates. Journal of General Virology. 1994;75:3541-3546.
23. Horser CL, Karan M, Harding RM, Dale JL. Additional Rep-encoding DNAs associated with banana bunchy top virus. Archives of Virology. 2001;146:71-86
24. Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE. Multiplex PCR: Optimization and Application in Diagnostic Virology. Clinical Microbiology Reviews. 2000;13:55 9-570
25. Nassuth A, Pollari E, Helmeczy K, Stewart S, Kofalvi SA. Improved RNA extraction and one-tube RT-PCR assay for simultaneous detection of control plant RNA plus several viruses in plant extracts. Journal of Virological Methods. 2000;90:3 7- 49.
26. Stellrecht KA, Woron M, Mishrik NG, Venezia RA. . Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. Journal of

Clinical Microbiology. 2004;42(4):1528-1533.
27. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution. 2021;38(7):3022–3027
28. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution. 2018;35(6): 1547.
29. Patel J, Kalaria RK. *In-silico* Analysis & homology of papaya ring spot virus & papaya leaf curl virus coat protein, Bulletin of environment, pharmacology and life science. 2018;7(8):19-28.
30. Kumar SP, Patel SK, Kapopara RG, Jasrai YT, Pandya HA. Evolutionary and Molecular aspects of Indian tomato leaf curl virus coat. International Journal of Plant Genomics. 2022;1-12
31. Solanki, Ravindra, Kalaria RK. Molecular identification and in silico characterization of coat protein in tomato leaf curl virus associated in tomato from South Gujarat Region of India. International Journal of Current Microbiology and Applied Sciences. 2019;8(07):456-466.
32. Li G, Piampongsant S, Faria NR, Voet A, Pineda-Pena AC, Khouri R, Lemey P, Vandamme A-M, Theys K. An integrated map of HIV genome-wide variation from a population perspective Retrovirology. 2015;12:18.
33. Belmabrouk S, Kharrat N, Abdelhedi R, Amine BA, Riadh B, Ahmed R. Screening of nucleotide variations in genomic sequences encoding charged protein regions in the human genome. BMC Genomics. 2017;18:588.
34. Prajapati R, Marwal A, Gaur RK. Recognition of errors in the refinement and validation of three-dimensional Structures of AC1 proteins of begomovirus strains by using ProSA-Web. Journal of Viruses. 2014;6.
35. Meena RK, Thakur S. *In-silico* Analysis and homology modelling of coat-protein of garlic common latent virus isolates from India. Indian Journal of Biotechnology. 2016;26(4):45-57.
36. Likhith RK, Peter A. Comparative *In silico* Analysis of Coat Protein (CP) of Tomato Leaf Curl Virus (ToLCV) and Tomato Yellow Leaf Curl Virus (TYLCV) and their Molecular Docking with GroEL Protein of Hamiltonella an Endosymbiont of their Vector *Bemisia tabaci*. Mysore Journal of Agricultural Sciences. 2023; 57(2):195.
37. Tian YP, Hepojoki J, Ranki H, Lankinen H, Valkonen JPT. Analysis of potato virus y coat protein epitopes recognized by Three Commercial Monoclonal Antibodies. PLoS ONE. 2014;9(12):3456-3478.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/113532