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Bioinformatics Analysis of the Interaction between Coat Protein and Nuclear Shuttle Protein in *Babuvirus*

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Abstract

Protein and protein interactions play important roles in many biological processes and are responsible for carrying out the function of biological regulatory network in living organisms. Previous study indicated that *Banana bunchy top virus* (BBTV) coat protein (CP) interacted with BBTV nuclear shuttle protein (NSP). However, the protein and protein interaction and the binding affinity of CP and NSP in *Babuvirus* are remaining unclear. In this study, the CPs and NSPs proteins of BBTV, *Abaca bunchy top virus* (ABTV) and *Cardamom bushy dwarf virus* (CBDV) were used for bioinformatic analysis. The binding free energy and the dissociation constant of the possible interaction proteins were tested in PPA-Pred2, and the results confirmed CP interaction with NSP in *Babuvirus*. The study will help us to understand the interaction between viral protein and viral protein, and the pathogenesis mechanism of *Babuvirus* in host plants.

Keywords

Babuvirus, Coatprotein, Nuclear Shuttle Protein, Interaction, Bioinformatics Analysis

1. Introduction

Banana bunchy top virus (BBTV) belongs to the genus *Babuvirus* within the family *Nanoviridae* [1] [2]. The BBTV-induced banana bunchy top disease (BBTD) can cause significant economic losses in banana industry [3] [4]. Its genome contains at least six circular single-stranded DNA molecules with 1.0 - 1.1 kb in size, which names DNA-R, -U3, -S, -M, -C, -N, respectively [5] [6]. Some

isolates may also carry 1 - 3 distant satellite molecules which have similar function as DNA-R component [7] [8]. Each component is encapsulated into an icosahedral particle of 17 - 20 nm that has no envelope [9]. BBTV is transmitted by the banana aphid, *Pentalonia nigronervosa* [10] [11].

DNA-S encodes a coat protein (CP) which encapsidates each DNA component inside [12]. Meanwhile, CP protein is a viral silencing suppressor that promotes the virus infection in host plants [13]. DNA-N encodes a nuclear shuttle protein (NSP) which can translocate the viral protein or protein and viral DNA complexes out of the cell nucleus [14]. Furthermore, the NSP protein contains an "FNGSF" motif that inhibits plant stress granules (SG) [15]. *In vitro* experiments showed that both CP and NSP proteins locate in the cytoplasm and nucleus of banana protoplast cell, and movement protein (MP) is able to re-locate NSP protein or NSP-DNA complex around the cell periphery, but not re-locate of the CP protein [14]. *In vivo* experiments showed the CP locates in the cell nucleus of *Nicotiana benthamiana*, while the NSP distributes in the cell nucleus and cytoplasm. Co-localization indicated that BBTV NSP interacts and re-distributes BBTV CP in tobacco cells [16].

According to the classification report of International Committee on Taxonomy of Viruses (ICTV, 2017), the family of *Nanoviridae* can be divided into the genus of *Babuvirus* and the genus of *Nanovirus* [1]. Currently, *Nanovirus* includes *Black medic leaf roll virus* (BMLV), *Faba bean necrotic stunt virus* (FBNSV), *Faba bean necrotic yellows virus* (FBNYV), *Faba bean yellow leaf virus* (FBYLV), *Milk vetch dwarf virus* (MVDV), *Pea necrotic yellow dwarf virus* (PNYDV), *Pea yellow stunt virus* (PYSV), and *Subterranean clover stunt virus* (SCSV), while *Babuvirus* includes BBTV, *Abaca bunchy top virus* (ABTV), and *Cardamom bushy dwarf virus* (CBDV).

Protein and protein interactions play important roles in many biological processes and are responsible for carrying out the function of biological regulatory network in living organisms. Previous study indicated that BBTV CP interacted with BBTV NSP [16]. However, the protein and protein interaction and the binding affinity of CP and NSP in *Babuvirus* are remaining unclear. In this study, the CPs and NSPs proteins of BBTV, ABTV, and CBDV were used for bioinformatic analysis. The study will help us to understand the interaction between viral protein and viral protein, and the pathogenesis mechanism of *Babuvirus* in host plants.

2. Materials and Methods

2.1. Materials

BBTV infected B2 sample (Haikou, China) was stored in Laboratory of Molecular Virology, Institute of Tropical Bioscience and Biotechnology (ITBB), Chinese Academy of Tropical Agricultural Sciences (CATAS). The complete nucleotide sequences of DNA-S (GenBank accession No. MG545612) and DNA-N (GenBank accession No. MG545615) were downloaded from the GenBank database

in National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov).

2.2. Nucleotide and Deduced Amino Acid Sequences of CP in *Nanoviridae*

The nucleotide sequences of BBTV *CP* (HQ616076.1), ABTV *CP* (FJ787435.1), CBDV *CP* (JX867540.1), FBNSV *CP* (GQ274036.1), PNYDV *CP* (KC979044.1), MVDV *CP* (LN890467.1), BMLV *CP* (NC_023304.1), FBNYV *CP* (GQ274028.1), FBYLV *CP* (HE654124.1) and PYSV *CP* (NC_023308.1) were downloaded from the GenBank database in NCBI. The amino acid sequences of BBTV CP (AEF97830.1), ABTV CP (ACN79534.1), CBDV CP (AGG38924.1), FBNSV CP (ACX50514.1), PNYDV CP (AHC72272.1), MVDV CP (CUR70740.1), BMLV CP (YP_008997802.1), FBNYV CP (ACX50506.1), FBYLV CP (CCF74114.1) and PYSV CP (YP_008997806.1) were downloaded from the GenBank database in NCBI as well. Sequence alignment was performed by using BioEdit (version 7.0.9.0). The sequence identities of the nucleotide and amino acid were analyzed by the Sequence Identity Matrix program in BioEdit.

2.3. Nucleotide and Deduced Amino Acid Sequences of NSP in *Nanoviridae*

The nucleotide sequences of BBTV *NSP* (FJ463047.1), ABTV *NSP* (NC_010314.1), CBDV *NSP* (JX867546.1), FBNSV *NSP* (GQ274038.1), PNYDV *NSP* (KC979047.1), MVDV *NSP* (NC_003643.1), BMLV *NSP* (NC_023301.1), FBNYV *NSP* (NC_003566.1), FBYLV *NSP* (HE654127.1) and PYSV *NSP* (NC_023310.1) were downloaded from the GenBank database in NCBI. The amino acid sequences of BBTV NSP (ACK43793.1), ABTV NSP (YP_001661656.1), CBDV NSP (AGG38930.1), FBNSV NSP (ACX50516.1), PNYDV NSP (AHC72275.1), MVDV NSP (NP_619764.1), BMLV NSP (YP_008997799.1), FBNYV NSP (NP_619573.1), FBYLV NSP (CCF74117.1) and PYSV NSP (YP_008997808.1) were downloaded from the GenBank database in NCBI as well. Sequence alignment was performed by using BioEdit (version 7.0.9.0). The sequence identities of the nucleotide and amino acid were also analyzed by the Sequence Identity Matrix program in BioEdit.

2.4. The Prediction of Interaction between CP and NSP Proteins in *Babuvirus*

Previous study indicated that BBTV CP interacted with BBTV NSP [16]. In order to determine the binding affinity of the two proteins, the amino acid sequences of BBTV CP (AEF97830.1) and BBTV NSP (ACK43793.1) were input into PPA-Pred2 (http://www.iitm.ac.in/bioinfo/PPA_Pred/index.html) for binding affinity analysis using jack-knife test [17]. The relationship between ABTV CP (ACN79534.1) and ABTV NSP (YP_001661656.1) and the relationship between CBDV CP (AGG38924.1) and CBDV NSP (AGG38930.1) were also conducted. The predicted value of Delta G (binding free energy) and the predicted value of

Kd (dissociation constant) were used to evaluate the interaction.

3. Results

3.1. Sequences Identity of Nucleotide and Amino Acids of CP in *Nanoviridae*

The complete ORF of BBTV *CP* is 513 bp, and its encoded protein is 170 aa with predicted molecular mass of about 19.35 kDa in size. Nucleotide sequence analysis showed that BBTV *CP* gene shares 34.5% - 97.8% identity with other plant viruses in *Nanoviridae* family. Among them, it shared 97.8% and 71.0% homology with ABTV and CBDV, respectively, while it was only 34.5% - 40.0% identity with *Nanovirus*. Amino acid sequences identity showed that BBTV CP protein is as high as 97.6% to ABTV, and 75.2% homologous to CBDV. However, the homology between the BBTV CP and *Nanovirus* CPs was less than 21.8% (Table 1 and Figure 1).

3.2. Sequences Identity of Nucleotide and Amino Acids of NSP in *Nanoviridae*

The complete ORF of BBTV *NSP* is 465 bp, and its encoded protein is 154 aa with predicted molecular mass of about 17.55 kDa in size. The sequence identity analysis of BBTV *NSP* gene showed that it both shares ~70% homology with ABTV and CBDV, and it was about 50% homology with *Nanovirus*. Amino acid sequence identity also found that it shares about 70% homology with ABTV and CBDV, while about 45% homology with *Nanovirus*, a little lower than nucleotide sequence identity (Table 2 and Figure 2). Furthermore, the "FNGSF" motif was conserved in *Nanoviridae* (Figure 2), which plays the role of inhibiting plant stress granules (SG) formation.

Table 1. Nucleotide and amino acid sequence identity analyses of BBTV *CP* gene with other *CP* genes of nanovirids.

Gene/Protein	BBTV CP	ABTV CP	CBDV CP	FBNSV CP	PNYDV CP	MVDV CP	BMLV CP	FBNYV CP	FBYLV CP	PYSV CP
BBTV CP	—	0.978	0.71	0.4	0.379	0.388	0.378	0.39	0.394	0.345
ABTV CP	**0.976**	—	0.717	0.402	0.381	0.387	0.384	0.383	0.385	0.347
CBDV CP	**0.752**	**0.764**	—	0.392	0.387	0.37	0.382	0.387	0.396	0.354
FBNSV CP	**0.218**	**0.218**	**0.224**	—	0.607	0.801	0.652	0.782	0.824	0.644
PNYDV CP	**0.218**	**0.218**	**0.235**	**0.558**	—	0.59	0.528	0.59	0.605	0.58
MVDV CP	**0.201**	**0.201**	**0.189**	**0.86**	**0.529**	—	0.644	0.793	0.815	0.653
BMLV CP	**0.2**	**0.2**	**0.205**	**0.568**	**0.431**	**0.586**	—	0.642	0.667	0.608
FBNYV CP	**0.195**	**0.195**	**0.195**	**0.848**	**0.529**	**0.819**	**0.551**	—	0.824	0.651
FBYLV CP	**0.183**	**0.183**	**0.183**	**0.883**	**0.523**	**0.837**	**0.586**	**0.889**	—	0.653
PYSV CP	**0.178**	**0.178**	**0.172**	**0.587**	**0.491**	**0.581**	**0.557**	**0.581**	**0.587**	—

Note: The bold numbers represent the amino acid sequence identity matrix; un-bold numbers represent the nucleotide sequence identity matrix.

Table 2. Nucleotide and amino acid sequence identity analyses of BBTV *NSP* gene with other *NSP* genes of nanovirids.

Gene/Protein	BBTV NSP	ABTV NSP	CBDV NSP	FBNSV NSP	PNYDV NSP	MVDV NSP	BMLV NSP	FBNYV NSP	FBYLV NSP	PYSV NSP
BBTV NSP	—	0.709	0.72	0.518	0.486	0.49	0.515	0.479	0.52	0.513
ABTV NSP	**0.701**	—	0.72	0.511	0.481	0.494	0.49	0.471	0.513	0.524
CBDV NSP	**0.703**	**0.677**	—	0.484	0.479	0.473	0.503	0.462	0.479	0.501
FBNSV NSP	**0.442**	**0.461**	**0.452**	—	0.714	0.829	0.766	0.833	0.88	0.79
PNYDV NSP	**0.423**	**0.442**	**0.414**	**0.751**	—	0.72	0.705	0.725	0.72	0.725
MVDV NSP	**0.461**	**0.48**	**0.452**	**0.888**	**0.751**	—	0.759	0.839	0.837	0.768
BMLV NSP	**0.448**	**0.442**	**0.426**	**0.803**	**0.718**	**0.803**	—	0.761	0.77	0.751
FBNYV NSP	**0.448**	**0.455**	**0.439**	**0.888**	**0.745**	**0.908**	**0.79**	—	0.839	0.766
FBYLV NSP	**0.455**	**0.461**	**0.452**	**0.908**	**0.738**	**0.908**	**0.79**	**0.895**	—	0.809
PYSV NSP	**0.461**	**0.474**	**0.433**	**0.816**	**0.738**	**0.823**	**0.79**	**0.823**	**0.843**	—

Note: The bold numbers represent the amino acid sequence identity matrix; un-bold numbers represent the nucleotide sequence identity matrix.

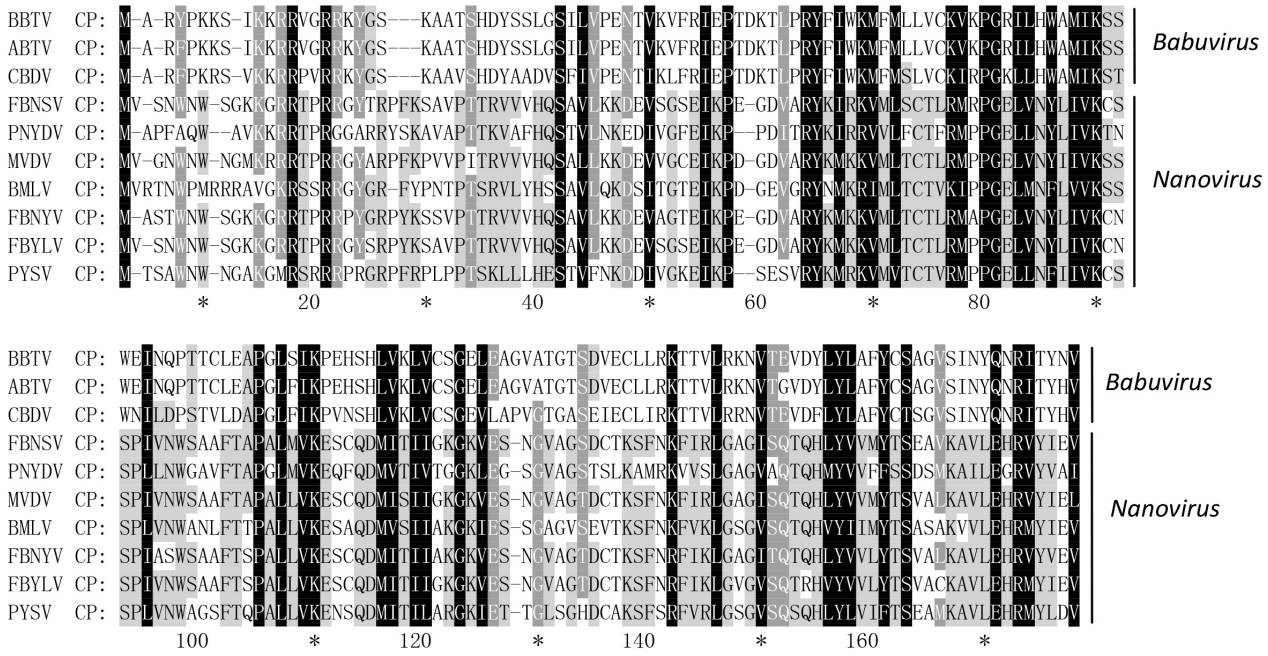

Figure 1. Multiple amino acid sequence alignment of BBTV CP with other CP of nanovirids. The black shaded regions indicate conserved residues, while the grey shaded regions are partially conserved residues with greater than 80% conservation. The numerical labeling on the residues indicated the total number of the amino acids at the corresponding position. GenBank accession numbers of the sequences used for the above analysis are as follows: BBTV CP, AEF97830.1; ABTV CP, ACN79534.1; CBDV CP, AGG38924.1; FBNSV CP, ACX50514.1; PNYDV CP, AHC72272.1; MVDV CP, CUR70740.1; BMLV CP, YP_008997802.1; FBNYV CP, ACX50506.1; FBYLV CP, CCF74114.1; PYSV CP, YP_008997806.1.

3.3. The Prediction of Interaction between CP and NSP Proteins in *Babuvirus*

PPA-Pred2 analysis indicated that BBTV CP had binding affinity with BBTV NSP. In detail, the predicted value of Delta G (binding free energy) was −11.69

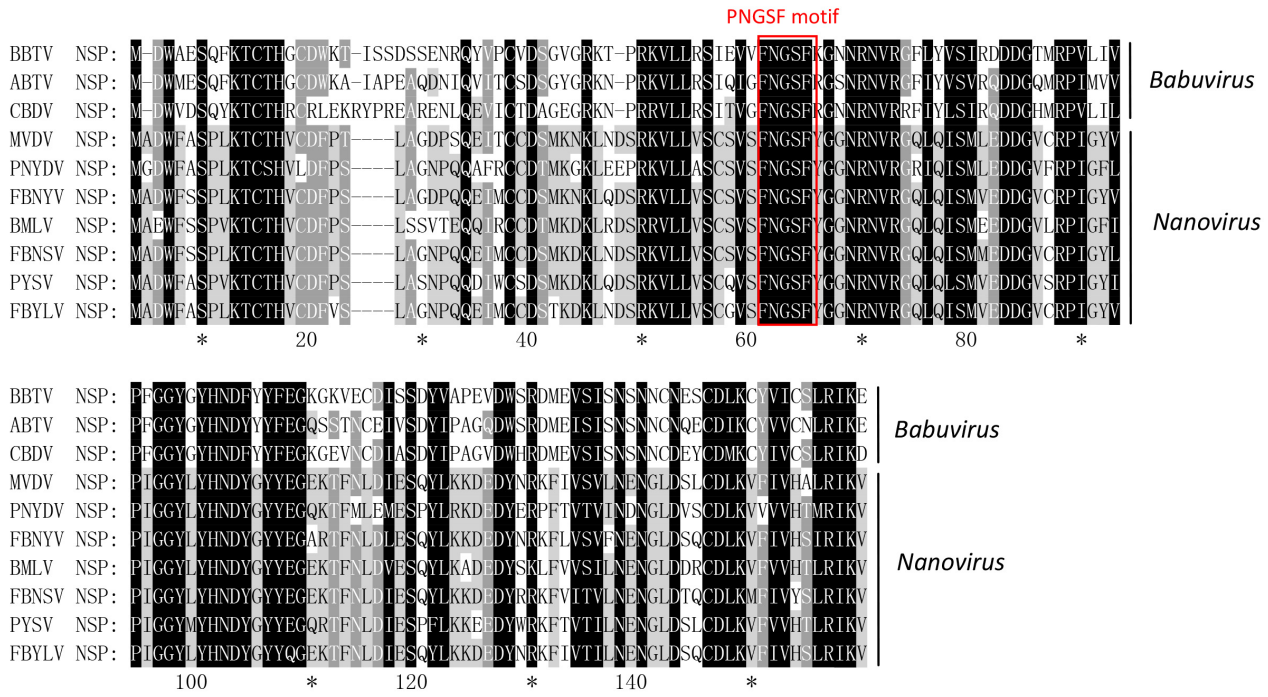

Figure 2. Multiple amino acid sequence alignment of BBTV NSP with other NSP of nanovirids. The black shaded regions indicate conserved residues, while the grey shaded regions are partially conserved residues with greater than 80% conservation. The numerical labeling on the residues indicated the total number of the amino acids at the corresponding position. GenBank accession numbers of the sequences used for the above analysis are as follows: BBTV NSP, ACK43793.1; ABTV NSP, YP_001661656.1; CBDV NSP, AGG38930.1; FBNSV NSP, ACX50516.1; PNYDV NSP, AHC72275.1; MVDV NSP, NP_619764.1; BMLV NSP, YP_008997799.1; FBNYV NSP, NP_619573.1; FBYLV NSP, CCF74117.1; PYSV NSP, YP_008997808.1.

kcal/mol, and the predicted value of Kd (dissociation constant) was $2.65e^{-09}$ M. Meanwhile, the bioinformatic analysis confirmed the interaction between ABTV CP and ABTV NSP. The predicted value of Delta G (binding free energy) was −11.99 kcal/mol and the predicted value of Kd (dissociation constant) was $1.61e^{-09}$ M. Lastly, the bioinformatic analysis confirmed the interaction between CBDV CP and CBDV NSP as well. The predicted value of Delta G (binding free energy) was −8.92 kcal/mol and the predicted value of Kd (dissociation constant) was $2.88e^{-07}$ M. Therefore, the result confirmed CP interaction with NSP in *Babuvirus*.

4. Discussion

Protein and protein interactions are crucial bridges for many biological processes involved in cellular signaling, immunity, cellular transport, etc. [18] [19] [20]. Experimentally, protein and protein interactions have been mainly studied with the yeast two-hybrid system, GST pull-down, co-immunoprecipitation (Co-IP), co-localization and so on. In addition, the fluorescence resonance energy transfer (FRET), isothermal titration calorimetry (ITC), surface plasmon resonance (SPR) and PPA-Pred2 provide the affinity of interacting proteins in terms of binding free energy change and dissociation constant [21] [22] [23], thereby adding a new dimension analysis of the task of protein and protein inte-

raction network. Previous study indicated that BBTV CP interacted with BBTV NSP. In this work, the possible interaction of CP protein and NSP protein of BBTV, ABTV and CBDV was further predicted based on their amino acid sequences. The results confirmed CP interaction with NSP in *Babuvirus* by PPA-Pred2 analysis.

The interaction between CP and NSP in *Babuvirus* was well predicted by PPA-Pred2 analysis based on the amino acid sequences, not by experiments. However, this method has a few limitations. For example, the protein whose structure is unclear or the protein which needs further processing modification could not be widely used in this method. In addition, the method cannot be used for prediction the binding affinity of more than two proteins.

In this study, the NSP is a vial nuclear shuttle protein, which would help viral proteins or viral nucleic acid transport outside of the nucleus by interaction with MP [14]. Furthermore, the "FNGSF" motif that inhibits plant stress granules (SG) was conserved in all nanovirids NSPs [15]. It is speculated that *Babuvirus* NSP proteins have similar functions. The product of the *Babuvirus CP* gene is an important structural protein that constitutes the virion. Meanwhile, CP protein is a viral silencing suppressor that promotes the virus infection in the host plant. BBTV NSP affects the BBTV CP distribution by co-localization analysis, suggesting that BBTV NSP interacts and re-locates the BBTV CP in tobacco cells. During the virus infection, *Babuvirus* NSP would re-locate the *Babuvirus* CP, which plays the key role of interaction between virus and host, such as inhibition the host antiviral activity by CP. Therefore, the interaction of CP and NSP viral proteins would play a very important role in the *Babuvirus* infection or systemic infection, and further studies should be conducted.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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