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Promiscuously replication of betasatellites; *in silico* study of interaction between betasatellite iteron-like sequence and Rep of helper geminiviruses

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Abstract

Betasatellites, single-stranded circular DNAs, are multifunctional agents associated with monopartite begomoviruses (family *Geminiviridae*), that act as symptoms determinant. Begomoviruses are replicated by species-specific interactions between the viral replication-associated protein (Rep) and iteron motifs at the upstream of the origin of replication (*ori*). In contrast, promiscuous replication of betasatellites could be supported by different geminiviruses. In this study, the interaction of *Cotton leaf curl Multan virus* (CLCuMuV, genus *Begomovirus*) and *Beet curly top virus* (BCTV, genus *Curtovirus*) encoded Rep proteins with the iteron-like sequence of betasatellite, *5′*-GAGGACC-*3′*, was investigated using *in silico* approaches. Nucleotide sequences of two Rep-encoding genes were obtained from the GenBank database, NCBI. Physicochemical characteristics of Rep proteins and their secondary and tertiary structures were predicted using the SOMPA tool and I-TASSER servers, respectively. The binding affinity of the best-predicted models of both proteins toward betasatellite iteron-like sequence was assessed using Docking simulations. The results represented reliable tertiary structures and showed structural similarity for Rep of different analyzed geminiviruses. Cluster analysis of HADDOCK revealed more total binding energy for CLCuMuV Rep toward the iteron-like sequence than BCTV complex. These *in silico* results confirmed the more trans-replication activity for relative geminiviruses in replication of betasatellite genomes. They emphasized the role of iteron-like sequences in interactions with the Rep of helper geminiviruses. Furthermore, targeting of identified activate sites within Rep protein structures to interact with betasatellite genomes could be considered as a control measure for begomovirus/betasatellite complexes.

Keywords: betasatellite, begomovirus, replication-associated protein, genome replication

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Introduction

The geminiviruses, *Geminiviridae*, are a family of small, non-enveloped plant infecting viruses with genomes containing one or two circular single-stranded DNA(s) (ssDNA) (Fiallo-Olivé et al., 2021). It is a common feature for all geminiviruses to have an intergenic region (IR) with an inverted repeat sequence capable of forming a stem-loop (hairpin) structure. There is a genus-specific conserved nonanucleotide sequence (5′-TAATATTAC-3′ or 5′-TAATATTAC-3′) within the loop of the hairpin structure region. Nonanucleotide is an active site for genome replication through rolling circle replication (RCR) by replication-associated protein (Rep) (Brown et al., 2012). Rep binds to the reiterated motifs (iterons) at the left position of the origin of replication (*ori*) within the IR. Replication of viral DNA initiates by introducing a nick into the genus-specific conserved nonanucleotide sequence by Rep (Behjatnia et al., 1998; Lin et al., 2003). Host DNA polymerase would bind to the developed free 3′-hydroxyl end generated by the Rep nuclease activity and triggers ssDNA synthesis. The synthesized ssDNA will convert to the dsDNA intermediate molecule and re-enter to the replication cycle (Jeske et al., 2001).

Among geminiviruses, the *Begomovirus* genus, with more than 400 assigned species is the biggest genus of viral taxonomy, till today (Fiallo-Olivé et al., 2021). Numerous economically important crops such as cotton, cassava, tomato, potato, and pepper are affected by these infective agents (Navas-Castillo et al., 2011). Begomoviruses are naturally transmitted by whitefly (*Bemisia tabaci* Gen.) in a persistent manner. Moreover, seed transmissibility of some destructive begomoviruses has been reported (Kim et al., 2015; Kil et al., 2016; Sangeetha et al., 2018). Genetically, begomoviruses are divided into two sub-groups of New World (NW), and Old World (OW) based on genome arrangement and phylogenetic studies (Nawas-ul-Rehman et al., 2009). Most bipartite begomoviruses are distributed in NW. OW begomoviruses are mostly monopartite and associated with different sub-viral fragments including alpha-, delta-., and betasatellites (Lozano et al., 2016). Satellites are sub-viral agents without functional genes that are required for replication. They are depended on their helper viruses to replicate during infection cycle (Briddon et al., 2012).

Betasatellites are small circular ssDNA which have been isolated from plants infected with certain monopartite begomoviruses (Briddon & Stanley, 2006). Betasatellites are responsible for the induction of disease symptoms in some host plants and play critical roles in determining the host range of helper begomoviruses, which could lead to the emergence of new complexes causing severe epidemics (Zhou, 2013). The genome of betasatellites is approximately 1350 nt in length that requires a helper geminivirus for replication, encapsidation, insect transmission, and movement within the plants (Zhou, 2013). Analyzed betasatellite sequences revealed a conserved organization consisting of a satellite-conserved region (SCR), an adenine-rich region, and a single complementary-sense open reading frame (ORF), *βC1* (Briddon & Stanley, 2006). The encoded betasatellite protein affects the helper begomovirus cycle by triggering disease symptoms, suppressing gene silencing pathways, and interacting with different cellular pathways and factors (Mosharaf et al., 2020a). Unlike species specific replication of helper geminiviruses, betasatellites can be trans-replicated by both cognate and non-cognate geminiviruses including *Cotton leaf curl Multan virus* (CLCuMuV, genus *Begomovirus*) and *Beet curly top virus* (BCTV, *Curtovirus* genus), with different efficacy (Kharazmi et al., 2012). Zhang et al. identified a novel sequence element termed Rep-binding motif (RBM), 5′-GAGGACC-3′, just upstream of the conserved region of betasatellite origin, which is responsible for binding Rep of different helper geminiviruses (Zhang et al., 2016).

Previously, we used a bioinformatics approach to clarify the interference phenomenon between the *Beet curly top virus* and *Beet curly top Iran virus* through a study on interactions between Rep protein molecules and viral nonanucleotide motifs (Tabein & Hemmati, 2022). The both of CLCuMuV/BCTV and also different betasatellite members have been reported from Iran (Mosharaf et al., 2020b; Bananej et al., 2021; Tabein & Hemmati, 2022). In the present study, to evaluate the support of different helper geminiviruses from betasatellite replication, we predicted the structures of the Rep proteins of the two begomovirus and curtovirus members. Furthermore, we estimated their interactions with the RBM by computational analysis based on docking simulation.

Significant advances in molecular biology, disease characterization, genomic technologies, and agriculture have led to explosive growth in the biological information generated by the scientific community. To manage this vast data, bioinformatics plays a significant role (Roy, 2013). The ultimate goal of bioinformatics is to answer questions arising from the genome revolution and find new biological insights by analyzing biological information (Zengyou, 2015; Hemmati, 2022). This study and further bioinformatics analysis may provide insight into how different geminiviruses acquire satellite molecules and trans-replicate them to overcome host defense mechanisms and express more severe symptoms.

Materials and Methods

Data retrieval and primary analysis of Rep proteins

The genome sequences of CLCuMuV (accession number NC_011804.1) and BCTV (accession number X97203.1) and the deduced amino acid sequences of Rep were obtained from the GenBank database of NCBI. A Similarity index of the sequences with other known Rep sequences retrieved from the GenBank database was estimated using the NCBI BlastP server (Altschul et al., 1997). Amino acid sequence alignment of the Reps of CLCuMuV and BCTV was carried out by CLUSTAL W (Thompson et al., 1994). The Expasy ProtParam server was used to further analysis of the physicochemical characterization. The secondary structure of both encoded Rep proteins was predicted by the SOPMA (Self-Optimized Prediction Method with Alignment) tool (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html).

Homology modeling of Rep proteins of CLCuMuV and BCTV

The retrieved sequences were analyzed by Iterative Threading ASSEmbly Refinement server (I-TASSER) to predict the structural models of the Rep proteins of CLCuMuV and BCTV (Zhang, 2008; Yang et al., 2015). The quality of the structures was assessed based on their confidence score (C-score), which estimates the models' global accuracy, TM-score, the topological similarity of the protein structures, and RMSD. RMSD is a quantitative measure of structural similarity between two or more protein structures (Pawlowski et al., 2008). The RMSD score between 1 and 2 Å represents closely related proteins, and TM-score between 0.5 and 1.0 indicates that the superimposed proteins may have a similar fold. Moreover, the C-score strongly correlates with the quality of the final models and is typically in the range of -5 to 2. At the same time, higher C-score values represent a model with high confidence. Moreover, the reliability of the predicted models of the two Rep proteins was assessed by Procheck, Verify-3D score, and Z-score (https://prosa.services.came.sbg.ac.at/prosa.php).

Docking analysis

The best Rep protein models obtained from I-TASSER was used in Docking analysis. Docking analysis was performed between the Reps of CLCuMuV and BCTV and RBM (5′-GAGGACC-3) (Zhang et al., 2016) using the

HADDOCK (High Ambiguity Driven protein-protein Docking) web server (de Vries et al., 2010; Kurkcouglu et al., 2018). With HADDOCK, the plausible residues contributing to the protein-nucleotide interface are either active, defined as the residues that make contact within the complex, or passive, known as the residues that potentially make contact. Firstly, all residues of Rep were defined as inactive. Moreover, all residues in the RBM motif were considered active residues, and passive residues were automatically determined by default in the program. The HADDOCK protocol consisted of 1000 rigid-body docking solutions followed by a semi-flexible refinement of the 200 best complex models in clear water. Using the HADDOCK default settings, conclusive selected structures were clustered based on RMSD criteria ranked based on averaged HADDOCK score. The CHIMERA software (version 1.14) obtained a superimposed view of the binding complex between Reps and the RBM motif in HADDOCK (Pettersen et al., 2004). A schematic view of the hydrogen bond interactions and nonbonded contacts between the nonanucleotide and the residues involved in the Rep binding site was obtained by the PDBsum server (de Beer et al., 2014).

Results

Primary and secondary structures of Rep proteins

The molecular weight (MW) of CLCuMuV encoded Rep with 362 amino acids (aa) was predicted to be 41 kDa with pI 6.47, indicating that the protein is acidic. The total number of negatively charged residues (Asp+Glu) (45) was higher than that of positively charged residues (Arg+Lys) (42). CLCuMuV encoded Rep had lower stability (<5 hours) than BCTV (>16 hours) due to the higher predicted instability index of 42.08, compared to 36.77 for BCTV (Idicula-Thomas & Balaji, 2005). Rep of BCTV encompassing 353 aa with MW of 40.2 kDa and pI 6.83. BCTV encoded Rep had a higher number of negatively charged residues (40) than positively charged residues (39), similar to CLCuMuV encoded Rep (Table 1).

The amino acid sequence of both intended Rep proteins aligned with the UniProt database showed 59.18% identity (Figure 1), suggesting possible structural differences and subsequently, different interactions capacity.

Table 1. Summary of primary structure analysis and secondary structure prediction for Rep proteins of CLCuMuV and BCTV.

Tools	Parameters	CLCuMuV	BCTV
ProtParam	Number of amino acids (aa)	362	353
	Molecular weight (MW)	41029.97	40207.28
	Theoretical isoelectric point (pI)	6.47	6.83
	Total number of negatively charged residues (Asp+Glu)	45	40
	Total number of positively charged residues (Arg+Lys)	42	39
	Instability index	42.08	36.77
	Aliphatic index	68.48	76.60
	GRAVY[a]	-0.688	-0.581
SOPMA			
	Alpha-helix (%)	32.32	33.14
	Extended strand (%)	16.85	16.43
	Beta-turn (%)	4.42	4.82
	Random coil (%)	46.41	45.61

```
BCTV  ---MPFYKKAKNFFLTYPQCSVTKEDALEQLLAINTPSNKKYIRICRELHDNGEPHLHAL 57
CLCuMuV MPSKRFQIYSKNYFLTYPKCSLTKEEALSQIQNLQTPTNKKFIKICKELHENGEPHLHVL 60
            *   :**:*****:**:***:**.*:  ::**:***:*:**:***:*******.*

BCTV  IQFEGKVQIRNARYFDLQHRSTSKQFHCNIQGAKSSSDVKSYVSKDGDHIDWGEFQVDGR 117
CLCuMuV IQFEGKYKCQNQRFFDLVSPTRSAHFHPNIQGAKSSSDVKDYIDKDGDTLEWGEFQIDGR 120
       ****** : :* *:***   : * :** ***********.*:.**** ::*****:***

BCTV  SARGGQQTANDAAAEALNAGNALEALQIIREKLPEKYIFQYHNLKPNLEAIFLPPPDLFQ 177
CLCuMuV SARGGQQTANDAYAAALNAGSKSEALRVIKELAPKDFVLQFHNLNANQSKIFQEPPAPYI 180
       *********** * *****.  ***::*:*  *:.:::*:***: * . **  **  :

BCTV  PPFPLSSFTRVPDIIQEWADSYFGLDPAAPFRYNSIIIEGDSRTGKTMWARCLGPHNYIT 237
CLCuMuV SPFSRSSFDQVPEELEVWAIDNVVDPAARPLRPRSIVIEGDSRTGKTMWARSLGPHNYLC 240
        **  *** :**: :: ** . .    * *:* .**:**************.******:

BCTV  GHLDFSLKTYSDNVLYNVIDDVDPNYLKMKHWKHLIGAQREWQTNLKYGKPRVIKGGIPS 297
CLCuMuV GHLDLSPKVYSNDAWYNVIDDVDPHFL--KHFKEFMGAQRDWQSNTKYGKPVQIKGGIPT 298
       ****:* *.**::. **********::*  **:*.::****:**:* *****  ******:

BCTV  IILCNPGEGSSYQDFLNKSENEALRSWTLQNSVFAKLTSPLFDNNQEASSQDQTSL---- 353
CLCuMuV IFLCNPGPHSSYKEFLDEEKNTALKNWAVKNAIFITLEGPLYSGTNQSTAQGSEEAHQEE 358
       *:*****  ***::**::.:* **:.*:::*::* .* .**:...::::::*.. .

BCTV  ----
CLCuMuV ESRS 362
```

Figure 1. Multiple sequence alignment of the amino acid sequences of the replication associated proteins of CLCuMuV (accession number: NC_011804.1) and BCTV (accession number: X97203.1) by CLUSTAL W software.

Table 2. The quality score of the predicted models, and estimated scores for predicted tertiary structures of CLCuMuV and BCTV.

Parameters	CLCuMuV	BCTV
C-score	-2.26	-2.36
TM-score	0.45	0.44
RMSD	11.9	12.1
Procheck (%)	68.00	65.2
Verfiy_3D (%)	79.83	76.77
Errat (%)	86.68	86.66
Z-score	-4.28	-2.85

The analysis of the secondary structures indicated that both proteins had the same structures containing random coils as the predominant element, 46.41 and 45.61% in CLCuMuV and BCTV, respectively. Alpha-helix and extended strand formed the elements with the highest frequency in predicted secondary structures of Rep proteins of CLCuMuV and BCTV, respectively, 32.32 and 33.14% for alpha-helix, and 16.85 and 16.43% for extended strand (Table 1).

Prediction of tertiary structures of CLCuMuV and BCTV encoded Rep proteins

I-TASSER predicted the tertiary structures of both intended Rep proteins (Figure 2). Models with the lower RMSD, TM-score, and C-score were selected as reliable models to use in HADDOCK analysis in interaction with the RBM motif. The scores of predicted models are listed in Table 2.

Based on Procheck scores, results showed that

68 and 65.2% of the Rep residues were placed in the most favored regions for CLCuMuV and BCTV, respectively (Table 2). VERIFY_3D indicated that 79.83 and 76.77% of residues in the Rep models of CLCuMuV and BCTV, respectively, have a score between 0.2 and 0.71. They can therefore be considered acceptable. The ERRAT scores were about 86.68 and 86.66% for CLCuMuV and BCTV, respectively (Table 2), showing that the overall quality of nonbonded interactions in the protein structures was appropriate. The Z-scores of both models were calculated to be about -4.28 and -2.85 for CLCuMuV and BCTV, respectively, similar to the values commonly found in the native structure of proteins (Table 2). These results indicate that the predicted models were reliable. Therefore, the best-fitted models were selected for docking analysis.

Relative geminiviruses support the trans-replication of betasatellites with more efficacy

Cluster analysis showed that CLCuMuV encoded Rep/RBM complex had more HADDOCK score (-49.8) than BCTV encoded Rep/RBM complex (-46.9) (Table 3). These results showed more affinity for CLCuMuV encoded Rep to bind with the iteron-like sequence of the betasatellite. This study indicated that non-relative helper geminivirus, BCTV, could support trans-replication of betasatellite with a lower affinity between Rep and RBM, as previously confirmed *in vitro* (Kharazmi et al., 2012).

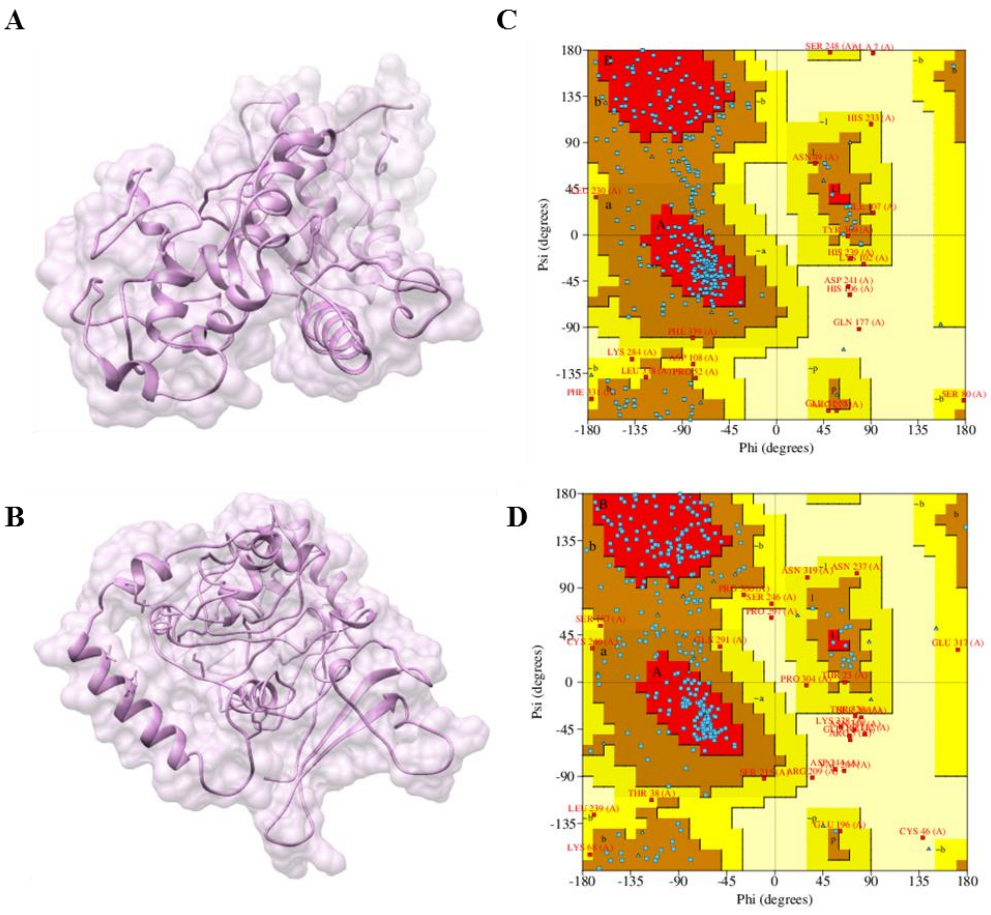

Figure 2. Ribbon representation of the structural models of Rep proteins from BCTV (A) and CLCuMuV (B). Validation of the structural models of Rep proteins from BCTV(C) and CLCuMuV (D) calculated by Ramachandran plot.

Table 3. Statistics of HADDOCK results for top-ranked cluster of different interactions between CLCuMuV and BCTV encoded Rep proteins with RBM motif.

Interaction	Cluster rank	HADDOCK score	Cluster size	RMSD	Z-score	Energy (Kcal.mol^{-1})			Buried surface area (Å^2)
						Van der Waals	Electrostatic	Desolvation	
CLCuMuV-RBM	1	-49.8	12	2.2	-1.7	-20.10	-121.2	-6.4	802.30
BCTV-RBM	2	-46.9	9	2.7	-1.6	-18.80	-92.7	-9.6	675.50

To further investigate the interactions between Reps and the RBM motif, three-dimensional structures of these interactions were obtained with HADDOCK and the CHIMERA software (Figure 3). Hydrogen bonding and van der Waals interactions between the RBM motif and amino acid residues of Rep proteins, which were directly interacting with RBM, were estimated by the PDBsum server (Figure 3). This analysis showed that hydrogen bonds were more frequent in the interaction between the amino acid residues of CLCuMuV encoded Rep with the RBM motif (Figure 3A). Moreover, CLCuMuV encoded Rep complex contained more hydrogen bond contacts than BCTV Rep. Lys 150 and His 162 were the most frequent amino acid residues of Rep proteins involved in binding the RBM motif.

Discussion

Betasatellites, as begomoviruses dependent small circular single-stranded DNA, are multifunctional agents. They induce disease symptoms, suppress gene-silencing pathways and interact with different cellular pathways and factors. These sub-viral elements have a conserved genome organization that encodes just one functional open reading frame on the complementary-sense strand, βC1 (Mosharaf et al., 2020a). In contrast to the strictly specific replication of genomic DNA of begomoviruses, a betasatellite may associate with more than a single helper geminivirus (Dry et al., 1997; Kon et al., 2009; Zhou, 2013). In comparison, trans-replication of the betasatellite also exhibits a certain level of specificity; not all begomoviruses replicate a betasatellite or replicate it with equal efficiency (Sounders et al., 2008; Kharazmi et al., 2012). Accordingly, despite the apparent replication promiscuity, the simultaneous association of two distinct betasatellites with a single helper begomovirus isolate has rarely been reported in the field. Thus, the helper virus-mediated preferential replication of the cognate betasatellite may function to limit the coinfection of betasatellites and genome reassortment. Previously, several efforts were made to localize the sequence region required for betasatellite replication, and these collectively identified the sequence from the A-rich region to the SCR (Lin et al., 2003; Li et al., 2007; Saunders et al., 2008; Eini et al., 2009; Eini et al., 2016). However, the critical *cis* elements necessary for recognizing of the satellite *ori* and the mechanisms by which replication promiscuity and specificity are achieved have not been identified (Xu et al., 2019).

Previous studies showed the presence of iteron-like sequences in different betasatellites which was mapped at the upstream of SCR regions (Zhang et al., 2016; Xu et al., 2019). Many betasatellites have evolved to acquire homologous iteron-like sequences for efficient replication mediated by related helper viruses. However, mixed infections of different geminiviruses in a single host have been frequently reported in the field. They are a possible reason for acquiring a betasatellite by other geminivirus species. Therefore, in this study, we evaluate the efficacy of different supporting geminiviruses, a begomovirus and a curtovirus, in trans-replication of a single betasatellite molecule via interaction with an iteron-like sequence.

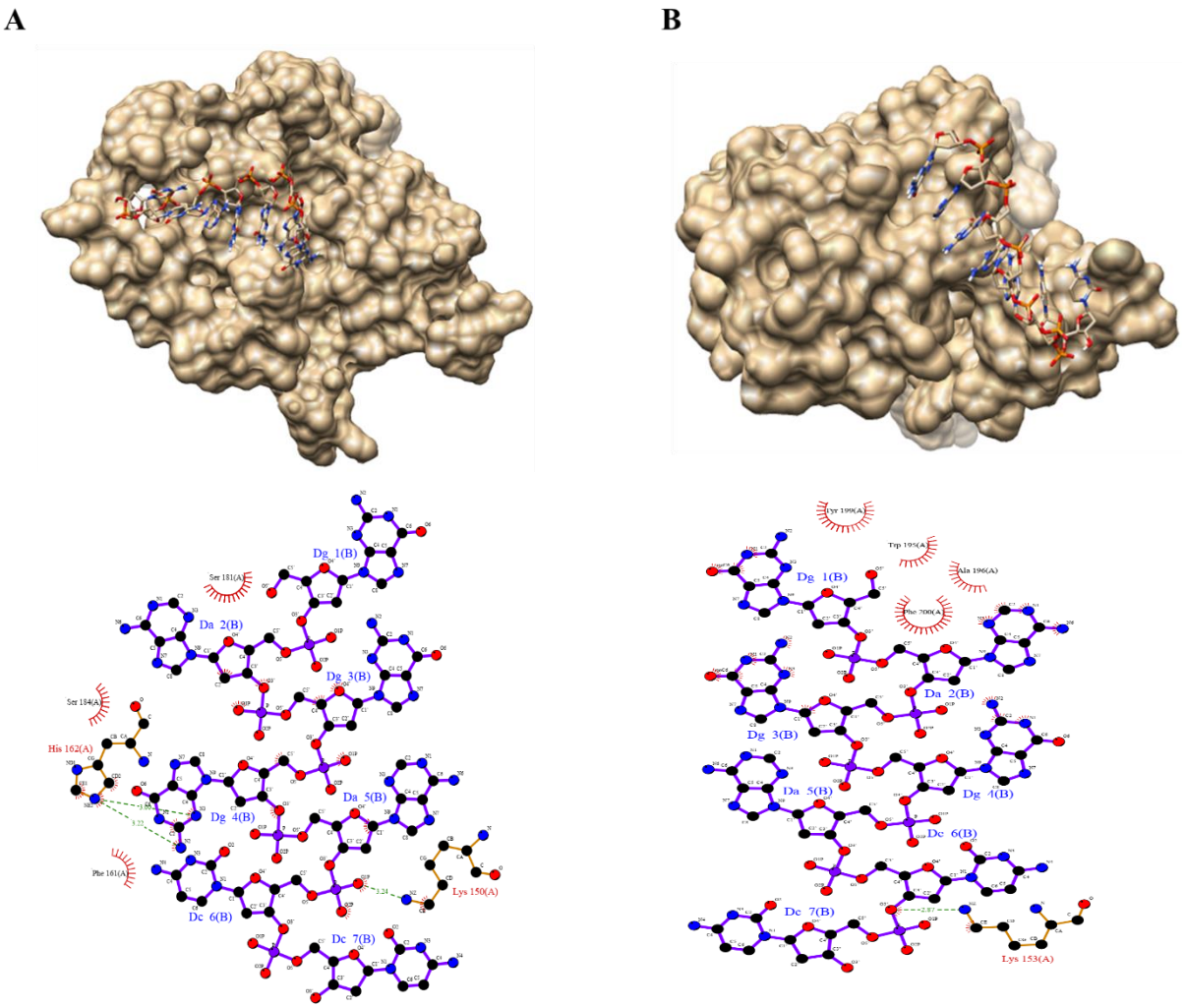

Figure 3. Representation of Rep/RBM interactions. Superimposed view of the binding complex between the CLCuMuV (A) and BCTV (B) encoded Rep proteins and the RBM motif obtained with HADDOCK and CHIMERA software.

The secondary structure of Rep proteins had similar frequency for different elements, while they showed low sequence identity, 59.18%, in their amino acid sequences (Figure 1). As a result of the low sequence identity, different tertiary structures and interaction capabilities were suggested. Therefore, the tertiary structures of both Rep proteins were predicted using I-TASSER. The reliable predicted tertiary models of Rep proteins were run through molecular docking experiments in interaction with the RBM motif. The docking analysis revealed more HADDOCK scores for relative helper begomovirus than non-relative curtovirus. In comparison with BCTV/RBM, CLCuMuV Rep contains more hydrogen bonds between amino acid residues and the RBM motif, resulting in a higher HADDOCK score (Table 2, Figure 3). It was in agreement with previous *in vitro* and *planta* experiments (Mubin et al., 2009). The obtained *in silico* results suggest that the RBM motif with iteron-like nucleotide sequence is a critical factor that could explain the different efficacy of relative and non-relative helper geminiviruses in trans-replication of different betasatellites.

Replication of betasatellites with different broad host range geminiviruses, makes them a

putative considerable symptoms determinant in the large number of host plant species. Additionally, the obtained *in silico* results are applicable to describe complex interactions during geminivirus infections. Nonetheless, the affinity binding of Rep/RBM in another geminivirus/betasatellite complexes must be further evaluated by *in vitro* analysis, including electrophoresis mobility shift assay.

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تکثیر غیراختصاصی بتاستلایت‌ها؛ مطالعه درون‌رایانه‌ای در خصوص برهمکنش بین توالی شبه ایترون بتاستلایت با پروتئین همراه با همانندسازی جمینی‌ویروس‌های کمکی

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چکیده

بتاستلایت‌ها مولکول‌های دی‌ان‌ایِ تک‌لایِ حلقوی مرتبط با اعضای جنس بگوموویروس (تیره Geminiviridae) هستند. بر خلاف همانندسازی ژنوم بگوموویروس‌ها که از اختصاصیت بالای گونه‌ای برخوردار است، گونه‌های مختلفی از تیره *Geminiviridae* قادر به حمایت از تکثیر ژنوم بتاستلایت‌ها هستند. در این مطالعه، برهمکنش پروتئین‌های همراه با همانندسازی کدشده توسط *Cotton leaf curl Multan virus* (CLCuMuV، جنس *Begomovirus*) و *Beet curly top virus* (BCTV، جنس *Curtovirus*) با نواحی شبه ایترون شناخته شده در توالی ژنوم بتاستلایت در شرایط درون‌رایانه‌ای مورد ارزیابی قرار گرفت. برای این منظور، توالی ژن‌های کدکننده پروتئین‌ها از بانک ژن دریافت شد. ضمن بررسی هم‌ترازی توالی‌های آمینواسیدی، ویژگی‌های ساختار درجه دوم پروتئین‌ها با استفاده از ابزارهای ProtParam و SOPMA مورد ارزیابی قرار گرفت. ساختار درجه سوم پروتئین‌های مورد نظر با استفاده از I-TASSER server تخمین زده شد. برازنده‌ترین مدل حاصل برای هر کدام از پروتئین‌ها، با استفاده از HADDOCK web server در برهمکنش با توالی شبه ایترون (5′-GAGGACC-3′) موجود در ژنوم بتاستلایت قرار داده شد. نتایج به دست آمده ضمن ارائه ساختار درجه سوم قابل اعتماد برای پروتئین‌های مورد بررسی، شباهت این ساختار را در پروتئین‌های همراه با همانندسازی گونه‌های متعلق به جنس‌های مختلف تیره *Geminiviridae* نشان داد. بررسی نتایج برهمکنش نشان داد که پروتئین همراه با همانندسازی گونه CLCuMuV از تمایل بالاتری در برهمکنش با توالی شبه ایترون بتاستلایت برخوردار است، در حالی‌که پروتئین کدشده توسط گونه BCTV نیز قادر به اتصال به توالی هدف بود. این نتایج ضمن تأیید پتانسیل تکثیر بالاتر بتاستلایت توسط ویروس‌های مرتبط، بر نقش توالی‌های شبه ایترون در برهمکنش با پروتئین‌های همراه با همانندسازی ویروس‌های کمکی تأکید می‌نماید. علاوه بر این، محل‌های فعال شناسایی شده در پروتئین‌های همراه با همانندسازی که در برهمکنش با ژنوم بتاستلایت نقش دارند، می‌توانند به عنوان هدفی در رویکردهای کنترل آلودگی ناشی از کمپلکس‌های بگوموویروس/بتاستلایت در نظر گرفته شوند.

کلیدواژه‌ها: بتاستلایت، بگوموویروس، پروتئین همراه با همانندسازی، همانندسازی ژنوم

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