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Research Article

In silico interaction studies of AV2 begomovirus and βC1 proteins of betasatellite with host *Capsicum annuum* proteins

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ABSTRACT

Disease complexes caused by geminiviruses have the potential to disrupt plant physiology and have severe consequences for a variety of economically significant crops around the world. The epidemic challenge for global food security is increased by the diverse geminivirus betasatellite interactions. Our previous study showed that the pathogenic response of DNA -A is enhanced in the association of betasatellite monopartite on chilli plants. Here, we explored the interactions of AV2, βC1 and AV2-βC1 complex viral proteins with eleven chilli host proteins; Helix-loop-helix transcription factor (MYC2), Ubiquitin Conjugating Enzyme, Asymmetric Leaves 1 (AS1), S-adenosyl homocysteine hydrolase (SAHH), Catalase 2, RNA - Dependent RNA Polymerase 6 (RDR6), Suppressor of Gene Silencing 3 (SGS3), and S-phase kinase-associated protein 1 (SKP1) through protein model generation via homology modelling and molecular docking analysis. Our findings suggest that βC1 showed the highest interaction with MYC2 protein, AV2 with CaRDR1 and AV2-βC1 with CaF1 as compared to other proteins.AV2-βC1 complex has the highest affinity towards all the host proteins and induces more pathogenic symptoms. We have provided the primary results of βC1 and host interaction, but its validation is required, which will be our further studies.

Keywords: Begomovirus, chilli, homology modelling, molecular docking

INTRODUCTION

Geminiviruses are plant DNA viruses; that belong to the Geminiviridae family. They cause destructive diseases and more than 95% yield loss of economically important crops (including monocotyledonous and dicotyledonous), weeds and ornamental plants around the globe (Marwal et al., 2013; Kumar, 2019). This family is classified into 14 genera based on genome-wide pairwise sequence identity, genome organization, host range and insect vector (Fiallo-Olive et al., 2021). Among all begomoviruses is one of the principal genera with more than 400 diverse species from tropical to subtropical regions and transmitted by the vector *Bemisia tabaci* (Whitefly) (Zerbini et al., 2017). DNA-A encodes 4-5 open reading frame ORF's in complementary sense direction (AC1/C1, AC2/C2, AC3/C3, AC4/C4) and two ORF's (AV1/V1, AV2/V2) in the virion sense direction. ORF's from AC1- AC4 produce replication-associated protein (Rep), transcriptional activator protein (TrAP), replication enhancer protein (REn) and AC4 protein, respectively while AV1-2 encodes coat protein and movement protein. These are all seven proteins directly involved in replication, movement, transmission, and

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pathogenesis (Ito et al., 2009; Roy et al., 2019). The monopartite remain in the association with three different satellites viz; alphasatellite, betasatellite and deltasatellites. Alphasatellite contains a single gene encoding alpha-rep protein in the virion-sense and play an important role in the epidemiology of begomovirus/ betasatellite complex (Briddon et al., 2018; Xie et al., 2010). Likewise, earlier findings revealed that betasatellite encodes for âC1 protein which determines pathogenicity of begomoviruses, involves in both transcriptional and post-transcriptional gene silencing suppression and conquers host defence activities of plants as a result of this disease severity enhances (Patil et al., 2010).

Spices are indispensable agents of Indian cuisine. Among all commonly used spices; green and red chilli (as whole and powdered form) plays an important role to enhance the flavour and aroma of the food. The active ingredient of the chilli is Capsaicin; which possesses uncountable health benefits viz; antioxidant, anti-mutagenic, anti-carcinomic and immunosuppressive activities. This economical crop has been introduced in India by the Portuguese in the Seventeenth century (Saxena et al., 2016). After that India has become major producer and exporter of this crop with 7.34 Lakh ha of the area under cultivation, 19.14 Lakh tonnes of total production and 2576 kg/ha of productivity. Among all states; Andhra Pradesh is the largest producer of chilli crop followed by Telangana, Madhya Pradesh, Karnataka, Odisha, Tamil Nadu, Maharashtra, Rajasthan, Gujrat, and Punjab (National Horticulture Board Database, 2020-21). Successful cultivation of this spice has declined due to various fungal, bacterial, viral diseases and insects. Among all, viral diseases seriously threaten chilli production and cause nearly 100 percent production loss of marketable fruit (King et al., 2011; Senanayake et al., 2012). A total of sixty-five plant viruses are associated with chilli plant. Out of those eleven viruses have been reported from India including Chi LCV, Cucumber mosaic virus (CMV), Pepper venial mottle virus (PVMV), Tobacco leaf curl virus (TLCV), Potato virus X (PVX), Potato virus Y (PVY), Tobacco ring spot virus (TRSV), Pepper vein bending virus (PVBV), Tomato leaf curl New Delhi virus (ToLCNDV), Chili mosaic virus and Capsicum chlorosis virus (Zehra et al., 2017; Devi et al., 2020). Mishra et al. (2020), have analysed the genome of begomovirus along with bipartite followed by agro-inoculation. It revealed that the infection clone of DNA-A alone developed mild symptoms in chili while severe symptoms of leaf curling, downward curling and vein yellowing were observed when DNA-A was co-inoculated with betasatellite. The âC1 protein alone or in complex with AV2 protein has more possibility to interact with the host protein and develop severe symptoms of leaf curl disease (LCD). To understand this hypothesis, current research work mainly focused on the study of individual and combined interactions of AV2 and âC1 viral proteins with eleven host proteins from chilli at *in silico* level. Several published literatures were considered to identify the host factors interacting with viral proteins chiefly emphasizing AV2 and beta C1 proteins as they are more involved in disease development. Though from the reported genes and their interacting host factors, the rationale for this study is to mine the actual three-dimensional structures, verified by experimental data that is available in the PDB database, for which we could identify eleven such structures at the beginning of this current study.

MATERIALS AND METHODS

Interaction studies of AV2 and βC1 with *C. annum* proteins

The protein-protein interaction study was conducted between host and begomovirus; includes retrieval of protein sequences from biological databases, model generation and docking analysis through bioinformatics software. Previous studies have shown the interactions of plant viral pathogenesis protein with host defence-related proteins (Glick et al., 2008; Shafiq et al., 2019).

Our previous work, Mishra et al. (2020) results of genome sequencing of isolates MM1, RV1, CS1, MM2, MM3 consisted of 2761, 2754, 2757, 1389, 1366 nucleotides respectively. All three MM1, RV1 and CS1 had structural similarity with monopartite begomoviral genomes (organization of the six ORFs that are AV1, AV2, AC1-AC4 and a conserved non-nucleotide sequence TAATATTAC) while MM2 had single ORF βC1, satellite conserved region and A-rich region and MM3 had three conserved domains, hairpin structure, alpha-rep and A-rich region. All sequences showed maximum sequence similarities with *Chili leaf curl virus* (ChiLCV), *Cotton leaf curl Multan virus* (CLCuMuV), *Tomato leaf curl Gujarat virus* (ToLCGV), *Chili leaf curl betasatellite* (ChiLCB) and *Cotton leaf curl Multan alphasatellite* (CLCuMuA) respectively. All sequences were submitted to NCBI under accession numbers MF737343, MF737344, MF737345, MF737346, and MF737349.

Here, in the present study, we have shown the docking of βC1 (MF737346) and AV2 (MF737343) proteins with various host proteins like Helix-loop-helix transcription factor (MYC2), Ubiquitin Conjugating Enzyme, Asymmetric Leaves 1 (AS1), S-adenosyl homocysteine hydrolase (SAHH), Catalase 2, RNA - Dependent RNA Polymerase 6 (RDR6), Suppressor of Gene Silencing 3 (SGS3), and S-phase kinase-associated protein 1 (SKP1). For constructing the 3D structures, a template for homology modelling was searched with Protein Data Bank (PDB) in NCBI BLAST. Proteins whose structure was not available in the databases, their sequences were retrieved from the NCBI protein database (https://www.ncbi.nlm.nih.gov/protein/) and were submitted to the online available Phyre 2 (Protein Homology/analogy Recognition Engine) server for developing 3D structures (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index). The file was converted in the .pdb format (Kelley et al., 2015) followed by docking studies using standalone Hex 8.0.0. Cuda software (Macindoe et al., 2010). Parameters taken into consideration during the docking process were: (1) Correlation type – Shape Only, (2) FFT Mode - 3D, (3) Post Processing- None, (4) Grid Dimension - 0.6, (5) Receptor range – 180, (6) Ligand range – 180, (7) Twist range –360 and (8) Distance Range – 40. The lowest E-value was considered for the structure generated in the PDB proteins for each protein molecule. The water molecules option was disabled and RMSD threshold was kept at 3.0 for better docking.

RESULTS

In the present study, the sequence of eleven *C. annum* proteins: RNA-dependent RNA polymerase 1 (CaRDR1), metacaspase 9 (Camc9), CCR4 associated factor1 (CaF1), Helix-loop-helix transcription factor (MYC2), Catalase isozyme 2, Ubiquitin-conjugating enzyme E2, *Capsicum annum* homeobox1 (CaHB1) nuclear factor, WRKY1 gene DNA binding transcriptional factor, S adenosyl homocysteine hydrolase (SAHH), *Capsicum annuum* transmembrane NAC transcription factor 1 (NAC1) and S phase kinase-associated protein 1 (SKP1) were retrieved from NCBI database and their respective 3D models were constructed.

To determine the possible interactions between AV2, βC1 and AV2-βC1 with the host protein, several binding affinity prediction methods were used. Receptor-ligand ellipsoidal view of protein-protein interactions with docking grids at the centre is presented in Figure 1-3. In Table 1, negative values of energy indicated that the degree of interactions between proteins is high. The best E-value (-660.30) was scored between βC1 and MYC2 host proteins as compared to other host proteins. Similarly, the interaction between AV2 and CaRDR1 (E-value; -593.97) protein was the best scored, followed by other capsicum proteins in the present study. Further, the molecular docking of the AV2-βC1 with the plant defence protein was also analysed. The analysis showed the highest affinity between AV2 + βC1- CaF1 with the lowest E-value at -721.16. Similarly, the best docking

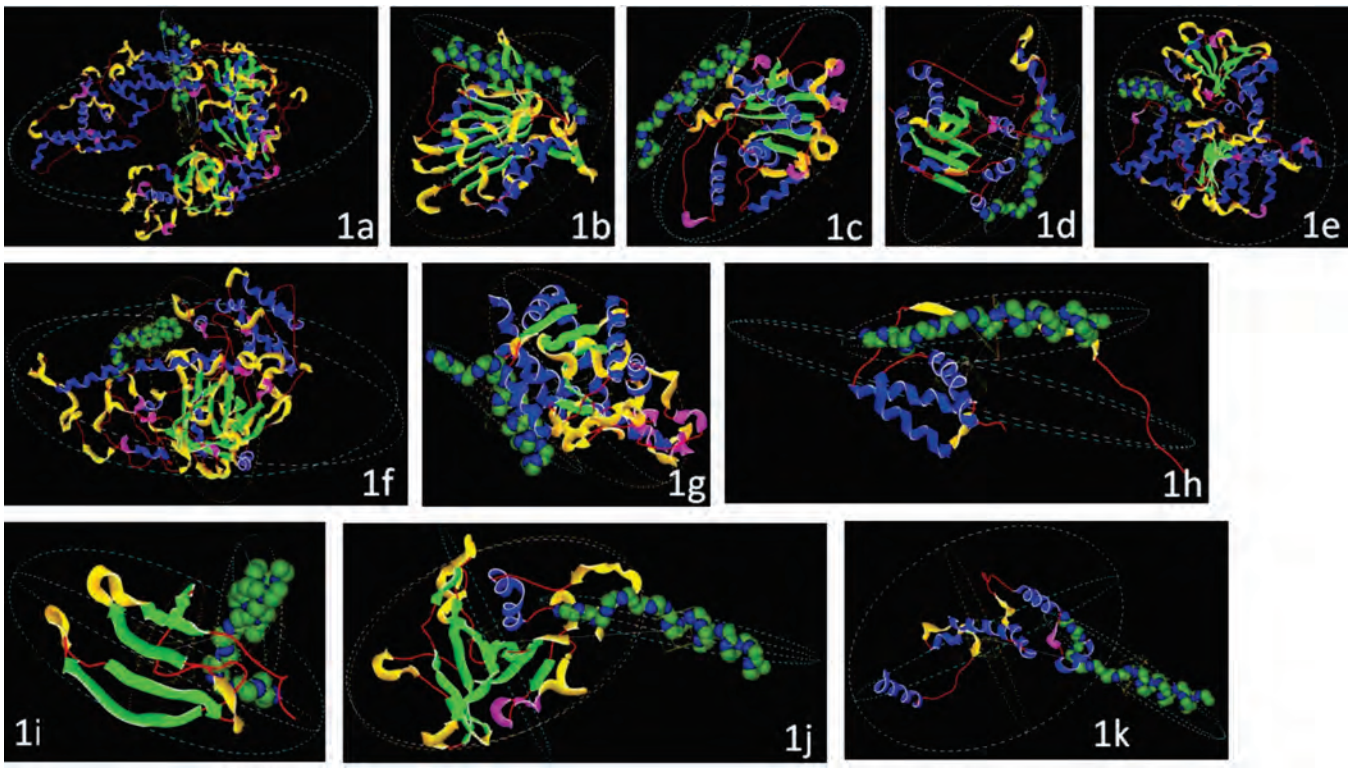

Figure 1: Receptor-ligand ellipsoidal view of host proteins-AV2 protein interaction with docking grids at the centre of the image. Interaction of AV2 protein with [a] *Capsicum annuum* CaRDR1 RDR1 mRNA for RNA dependent RNA polymerase 1, [b] *Capsicum annuum* metacaspase 9 mRNA, [c] *Capsicum annum* CCR4 associated factor1 CaF1, [d] *Capsicum annuum* Transcription factor MYC2, [e] *Capsicum annuum* S adenosyl homocysteine hydrolase, [f] *Capsicum annuum* Catalase isozyme 2, [g] *Capsicum annuum* ubiquitin conjugating enzyme E2, [h] *Capsicum annuum* homeobox1 CaHB1 nuclear factor, [i] *Capsicum annuum* WRKY1 gene DNA binding transcriptional factor, [j] *Capsicum annuum* transmembrane NAC transcription factor 1, [k] *Capsicum annuum* S phase kinase associated protein 1 SKP1

Table 1: E total / e-value of AV2 protein (ChiLCV; DNA-A) and βC1 protein (ChiLCB) with *Capsicum annuum* plant proteins.

S.No.	***Capsicum annuum* Host Proteins/Enzymes**	**E Total***		
		AV2	**βC1**	**AV2+βC1**
1	RNA dependent RNA polymerase 1 (CaRDR1)	**– 593.97**	-566.44	-597.62
2	Metacaspase 9 mRNA (CamC9)	– 578.65	-613.95	-364.18
3	CCR4 associated factor1 (CaF1)	– 558.28	-643.93	**-721.16**
4	Helix-loop-helix transcription factor (MYC2)	– 540.92	**-660.30**	-699.40
5	Catalase isozyme 2	– 536.23	-607.71	-643.06
6	Ubiquitin conjugating enzyme E2	– 534.47	-551.75	-236.49
7	Homeobox1 CaHB1 nuclear factor (CaHB1)	– 486.58	-454.03	-590.20
8	WRKY1 gene DNA binding transcriptional factor	– 454.31	-622.53	**-720.59**
9	S adenosyl homocysteine hydrolase (SAHH)	– 383.62	-586.61	-500.85
10	Transmembrane NAC transcription factor 1	– 372.04	-517.11	-527.84
11	S phase kinase associated protein 1 (SKP1)	– 262.16	-597.69	-342.92

*****low E total is directly proportional to a more stable protein complex.

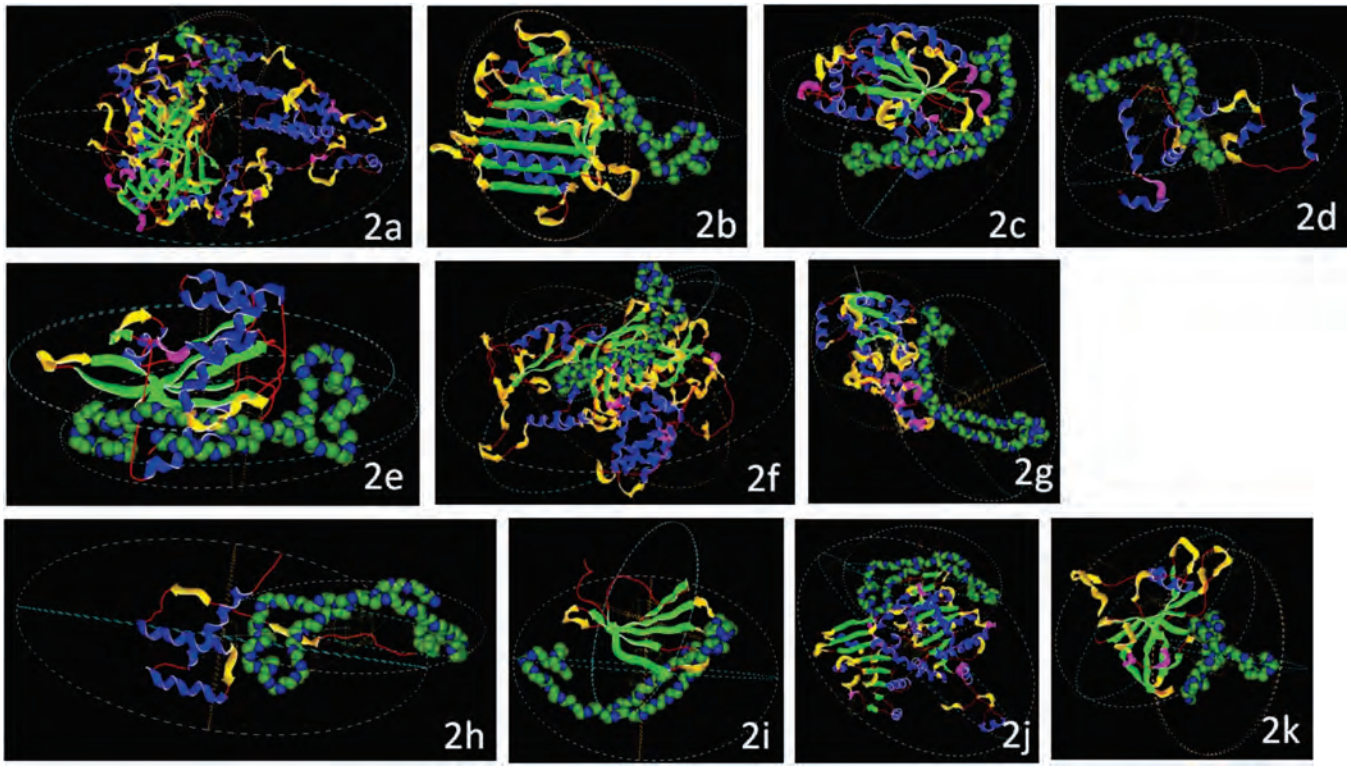

Figure 2: Receptor-ligand ellipsoidal view of host proteins- βC1 protein interaction with docking grids at the centre of the image. Interaction of βC1 protein with [a] *Capsicum annuum* CaRDR1 RDR1 mRNA for RNA dependent RNA polymerase 1, [b] *Capsicum annuum* metacaspase 9 mRNA, [c] *Capsicum annuum* CCR4 associated factor1 CaF1, [d] *Capsicum annuum* S phase kinase associated protein 1 SKP1, [e] *Capsicum annuum* Transcription factor MYC2, [f] *Capsicum annuum* Catalase isozyme 2, [g] *Capsicum annuum* ubiquitin conjugating enzyme E2, [h] *Capsicum annuum* homeobox1 CaHB1 nuclear factor, [i] *Capsicum annuum* WRKY1 gene DNA binding transcriptional factor, [j] *Capsicum annuum* S adenosyl homocysteine hydrolase, [k] *Capsicum annuum* transmembrane NAC transcription factor 1

interactions were recorded between ChiLCB-βC1 and the basic helix-loop-helix transcription factor MYC2. Another protein, WRKY1 transcriptional factor with E-value of -720.59 may also be considered as an important interaction with AV2- βC1 during the virus infection. Both viral proteins AV2 and βC1 individually and in the complex form a less stable protein complex with SKP1, CaHB1 and Ubiquitin conjugating enzyme E2 respectively as their E value is high. Results revealed that the docking score of host proteins with AV2-βC1 complex has a low E value with exception of three interactions. Ubiquitin-conjugating enzyme E2- AV2-βC1, (SKP1)- AV2-βC1 and CamC9- AV2-βC1; while high E value was scored when host proteins were docked with individual viral proteins.

DISCUSSION

The βC1 as well as the AV2 protein of geminivirus suppress the host defence mechanism system such as PTGS, TGS, ubiquitin-proteasome and defence hormones of the plants, thus increasing the begomovirus pathogenesis (Sharma et al., 2008; Zhou, 2013). Similarly, previous work by Gnanasekaran et al. (2019), reported that the non-specific binding of plant defence protein, *Nicotiana benthamiana* oxygen-evolving enhancer protein 2 (PsbP) with betasatellite protein enhances the pathogenicity and viral DNA accumulation. Further, findings by Nova and Jamsari (2020), suggested that ubiquitin-associated [UBA] and auto-inhibitory sequence [AIS] domains of Pepper-SnRK1 proteins from pepper

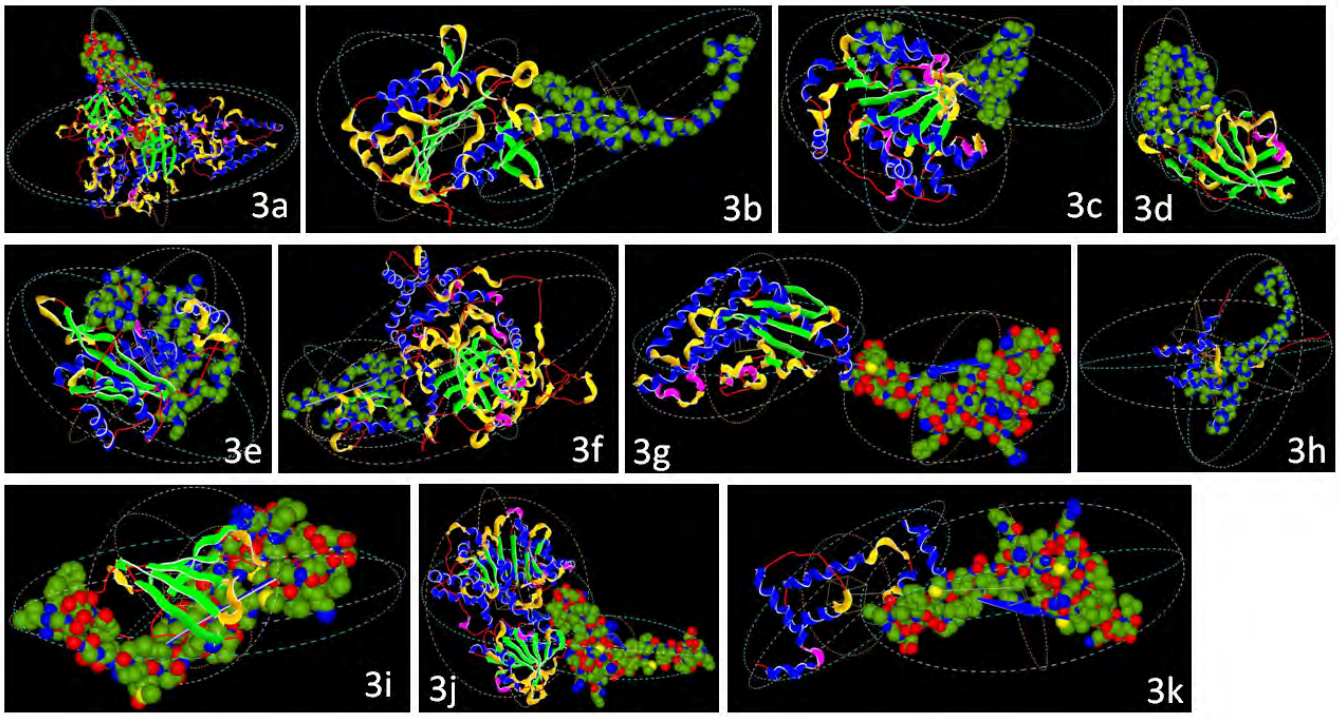

Figure 3: Receptor-ligand ellipsoidal view of host proteins- AV2 + βC1 protein interaction with docking grids at the centre of the image. Interaction of AV2 + βC1 protein complex with [a] *Capsicum annuum* CaRDR1 RDR1 mRNA for RNA dependent RNA polymerase 1, [b] *Capsicum annuum* metacaspase 9 mRNA, [c] *Capsicum annuum* CCR4 associated factor1 CaF1, [d] *Capsicum annuum* transmembrane NAC transcription factor 1, [e] *Capsicum annuum* Transcription factor MYC2, [f] *Capsicum annuum* Catalase isozyme 2, [g] *Capsicum annuum* ubiquitin conjugating enzyme E2, [h] *Capsicum annuum* homeobox1 CaHB1 nuclear factor, [i] *Capsicum annuum* WRKY1 gene DNA binding transcriptional factor, [j] *Capsicum annuum* S adenosyl homocysteine hydrolase, [k] *Capsicum annuum* S phase kinase associated protein 1 SKP1

are involved in the PepYLCV-βC1 interaction. Negative energy indicates higher affinity between the proteins as a more negative value in free energy is directly proportional to a more stable protein complex. Molecular docking interactions and molecular dynamics simulations of inhibitory peptides with viral DNA replication protein (Rep) showed stable structural features, which does not mean that peptides will interfere in viral replication (Ascencio *et al.*, 2021). The βC1-MYC2 interaction supports the earlier finding that βC1-MYC2 compromises the activation of regulated terpene synthase genes, resulting the improved performance of the vector for virus transmission, whereas; interaction of CaF1 with AV2+ βC1 suggests that suppression of CaF1 in chilli plants results in enhanced susceptibility and severe symptoms (Sarowar et al., 2007). MYC2 is thought to be important transcriptional factor for JA-regulated secondary metabolites synthesis such as terpene. The terpene is one of the important defence molecules by which plants defend themselves against the insects. Similarly, Li et al. (2014) demonstrated TYLCCNB C1 interaction with the basic helix-loop-helix transcription factor MYC2.CARDR1 is thought to limit the pathogen spread and suppress the plant virus replication and protect the plant (Qin et al., 2017). It is considered an important factor for the disease severity as it interferes with SA and JA signalling and enhances viral accumulation and symptoms (Jia et al., 2016). WRKY1 factor is associated with the upregulation of JA biosynthetic genes and increased JA production upon pathogen infection (Chen et al., 2019).

CONCLUSION

The tight interactions between begomoviral and chilli host proteins can result either in the activation or repression of activities of any of the interacting beings. In our studies all the interactions revealed above are in the high affinity column. Though only the tight interaction does not lead to any biological consequences absolutely, hence some negative controls can be used to ascertain such high-affinity interactions. We tried with some common molecules like benzene, phenol, acetate, etc., but were unascertained them being negative control due to their near to zero E values. Even though there are some host factors which were not reported for interactions still show some degrees of affinity, which might be the reason for being software with defined logarithms. But these 11 host factors showed very strong affinity, thus supporting the available literature. The major ones among the 11 selected host factors are RNA-dependent RNA polymerase 1 (CaRDR1), Helix-loop-helix transcription factor (MYC2) & WRKY1 gene DNA binding transcriptional factor. A molecular docking study of begomoviral proteins alone or in combination with several host proteins showed that AV2+ βC1 complex highest interaction with chilli host proteins as compared to individual interaction. This supports the present hypothesis that the pathogenicity of begomoviral proteins enhances with the satellite proteins. This computational-based study provides strategic begomovirus-chilli interaction in the host-pathogen interactome, which may be a foundation of functional analysis. Here we have provided the primary results of βC1 and host interaction, but its validation is required, which will be our further studies.

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Conflict of interest

All authors declare that there are no conflicts of interest associated with the manuscript.

Authors contribution

AM collected the data and performed computational analysis. AM and KJ collected the literature, wrote and edited the first draft of manuscript. RKG conceptualized, designed the work and made intellectual contributions in critical reviewing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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