In-silico Analysis of insecticidal proteins Cry1Ac, Cry2Ab and Cry1F against Lepidopteran pests using Molecular Docking studies

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Abstract:

Transgenic technology has brought a revolution in the development of insect resistant Bt cotton. Several insecticidal crystalline proteins have been characterised from Bacillus thuringiensis which had a potential role in combating insect pest, predominantly lepidopteran. In general, the cry proteins have greater toxicity and high affinity against a broad range of insect receptors. In the current study, we choose novel Bt proteins such as Cry1Ac, Cry2Ab and Cry1F to analyse through molecular docking against cadherin proteins of Helicoverpa, Spodoptera and Pectinophora. Structural bioinformatics is concerned with computational approaches to predict and analyze the spatial structure of proteins by using homology modeling. The structural prediction and interaction of bacterial three cry proteins and lepidopteran receptors (cadherin, cadherin like receptors) were chosen in this study. Molecular docking analysis revealed that the active residues of Cry1Ac protein were SER548, ARG657, HIS825, THR654,GLU597 and of Cry1F protein were ARG851, , THR625, GLN650 while that of Cry2Ab protein were HIS713, GLN650, ASP490, THR33, SER30. These residues of the Cry proteins are involved in the interaction with the insect cadherins. This study reveal the feasible interactions between cry toxins and cadherins ,which are formed H- bonding and hydrophobic interactions. These findings are suggested the basis for a broad spectrum efficacy of the Cry proteins against the Lepidopteran pest.

Key words: HAD DOCK, STML, Protein Modeling, Cry proteins, Cadherins.

1. Introduction:

Cotton (*Gossypium hirsutum)* is an important fiber crop cultivated in more than 100 countries in the world. It has been extensively used in textile industry, seed oil, paper, fertilizer and livestock feed etc (Palle *et al*., 2013). Cotton is severely affected by the incidence of boll worms and sap sucking insects. Researchers have generated transgenic cotton against insects by transferring insecticidal cry genes. The insecticidal proteins are crystalline (cry) inclusion, parasporal bodies, delta endo toxins formed during the sporulation of *Bacillus thuringiensis*. Cry proteins are effective against specific insect species including *Lepidopteran, Coleopteran, Hymenopteran, Dipteran and Nematode.* Cry toxins ingested by susceptible insects, cause death The Cry proteins are protoxins, that become active through cleavage by insect enzymes in the alkaline gut juice (pH 8-10) (Bravo *et al*., 2007). These toxins bind to specific receptors on the brush border membrane of the epithelial cells of the midgut make pores and penetrate into the cells and become swollen until cells lyse. The alkaline gut juice is now released into the hemocoel through pores that lead to pH rise and paralysis and death of the insect (Soberon *et al*., 2010).

Insecticidal cry proteins are important choice for controlling insect pest. The Cry toxins are produced by soil bacterium *Bacillus thuringiensis* during sporulation and used to control wide variety of agriculturally important insect pests (Bravo *et al*., 2011). The most economically important and destructive insect pest is the lepidopteran. Cry 1A group of proteins is toxic to insect larvae of Lepidopteran (Tabashink *et al*., 2013) and specifically toxic to insect larvae in the order *Lepidoptera* (Chen *et al*., 2007). The mode of action of Cry1A toxin involves in an interaction with membrane receptors (Chen *et al.*, 2007 & Bravo *et al.*, 2008). There are 4 different types of receptors in midgut specific to cry toxins such as cadherin and cadherin like proteins, APN (Amino peptidase), Alkaline peptidase and ABC transporters (Pardo Lopez *et al*., 2013 and Tanaka *et al*., 2013).

Cadherin like proteins are important for understanding the Bt insecticidal activity at molecular

level. The mutations in cadherin proteins lead to disruption of interaction with cry toxins which are tightly linked with resistance to cry toxins (Fabrick *et al*., 2007). These functional receptors are identified in insect mid gut (Vadlamudi *et al*., 1993). In previous studies reported that the TBR regions of lepidopteran are cadherin receptors and appear to be located in the membrane proximal cadherin repeats (Gomez *et al*., 2001 and 2002, Pigott *et al*., 2007, Park *et al*., 2009).
Transgenic cotton expressing cry1Ac toxin showed resistance against tobacco budworm and pink bollworm. Bollgard-II was introduced in the year 2003 and denoted as the next generation Bt cotton, produced Cry1Ac and Cry2Ab toxins. The multiple genes Cry1Ac and Cry1F were combined and developed as wide strike hybrid in 2004. The Bollgard II with Cry1Ac and Cry2Ab are highly toxic to Lepidopteran pest (Stewart *et al.,* 2000 and Jackson *et al*., 2000). The 3rd generation of Bt cotton varieties have combined multiple genes and have potential role in comating insect pest such as Cry1Ac+Cry2Ac+Vip3A, and Twin Link Plus (Cry1Ab+Cry2Ac+Vip3Aa19), and Wide strike with 3 multiple genes (Cry1Ac+Cry1F+Vip3A) (Vyavhare *et al*., 2017). The interaction of multiple Cry genes with Cadherin through *in silico* approach would anticipate the role of the proteins against bollworms.
However one of the major constrains of Bt cotton development is the emergence of pest tolerance. Several strategies have been already proposed to express multiple cry genes targeting different insect spectra (Dammak *et al*., 2011, Wang *et al.,* 2012, Jiang *et al.,* 2016). Katta et al. (2020) demonstrated three cry proteins (Cry1Ac, Cry2Ab, Cry1F) under the regulation of different promoters and showed resistance against *Helicoverapa* and *Spodoptera* insects. The production of multiple toxins with specific promoters could achieve broad spectrum of resistance to multiple insects and also avoid the evolution of pest tolerance towards Cry toxins. Validation of broad spectrum efficacy of these multiple Cry toxins by demonstrating its molecular interaction with the consequent insect receptors would be valuable.

Bioinformatic tools are being efficiently used to study the various molecular interactions. Molecular modeling or protein structure prediction and Protein docking are elucidated to study the interactions between Cry proteins and receptors in the insects extensively demonstrated (Tajne *et al*., 2014, Ahmad *et al*., 2015 and Berry *et al*., 2017, and). This study would assist in the development of a stable platform to explain a broad spectrum efficiency of multiple toxins and study the interaction mechanism of cry toxin- receptor complex and cry toxin insecticidal activity.
Docking is a computational approach that virtually used to predict a complex of two binding macromolecules. Specific docking methods are available for different binding partners such as HAD DOCK 2.2 (Van Zundert *et al*., 2016). In this context the novel stacked and multiple insecticidal proteins Cry2Ab- Cry1F- Cry1Ac with high toxicity and affinity introduced in to Cotton Narasimha CV (Katta *et al.,* 2020) to control this board spectrum insect pest. The use of Cry proteins with broad spectrum efficiency are major tools for insect resistance management.

2. METHODOLOGY

The interaction between the cry genes proteins from *Bacillus thuringiensis* and the cadherin protein from Lepidoptera (*Helicoverpa*, *Pectinophora*, and *Spodoptera*) was studied using *in silico* methods. The structural coordination for Cry1Ac that existed in the Protein Data Bank with the PDB ID: 4W8J was used for the analysis.

2.1Protein Structure Modeling

Template search with BLAST and HHBlits has been performed against the SWISS-MODEL template library (SMTL). The target sequence was searched with BLAST against the primary amino acid sequence contained in the SMTL. An initial HHblits profile has been built using the procedure outlined in HH3 Suit protein annotation (Steinegger *et al*., 2019) and followed by an interaction of HHblits against Uniclust30 (Mirdita *et al*., 2017). The resulted profile has then been searched against all profiles of the SMTL. Models are built on the basis of target-template alignment using ProMod3. Coordinates that are conserved between the target and the templates are copied from the template to the model. Insertions and deletions are remodelled using a fragment library and side chains are then rebuilt. Finally, the geometry of the resulting model was regularized by using a force field. In case loop modelling with ProMod3 fails, an alternative model is built with PROMOD-II (Guex *et al*., 2009). The global and per-residue model quality has been assessed using the QMEAN scoring function (Studer *et al.,* 2020). The quaternary structure annotation of the template is used to model the target sequence in its oligomeric form. It is based on a machine learning algorithm, Support Vector Machines (SVM), which combines interface conservation, structural clustering, and other template features to provide a quaternary structure quality estimate (QSQE) (Bertoni *et al*., 2005). The QSQE score is between 0 and 1, reflecting the expected accuracy of the inter-chain contacts for a model built on the basis on a given alignment and template.

Higher numbers indicate higher reliability. This complements the GMQE score, which estimates the accuracy of the tertiary structure of the resulting model.

For the protein structure modeling of Cry1F and Cry2Ab from Bt and Cadherin (CAD) and Cadherin-like protein (BtR) from Lepidoptera, their primary amino acid sequences were retrieved in FASTA format from UniProtKB. Their UniProt ID is as follows: Cry1F - B2ZPN5; Cry2Ab - P21254; Cadherin - A0A678NBJ2 from *Helicoverpa zea*, A0A060BFA9 from *Pectinophora gossypiella*, S5VXI8 from *Spodoptera exigua*; Cadherin-like protein - Q19KJ3 from *Helicoverpa armigera*, Q86DU3 from *Pectinophora gossypiella*, A0A0F6PPD3 from *Spodoptera litura*. The sequences were submitted to the Swiss-Model workspace in FASTA format. Models for each protein were generated based on the templates having sequence identity with the target proteins. After the models were generated, they were validated using Ramachandran plots and MolProbity. A Ramachandran plot is a way to visualize energetically favoured regions for backbone dihedral angles against amino acid residues in the protein structure. To determine the contours of favoured regions, data were extracted from 12,521 non-redundant experimental structures (pairwise sequence identity cutoff 30%, X-ray resolution cutoff 2.5Å) as culled from PISCES. Histograms with a binning of 4 degrees were then used to count Φ (Phi; C-N-CA-C) / Ψ (Psi; N-CA-C-N) occurrences for all displayed categories. The number of observed Φ / Ψ pairs determines the contour lines. MolProbity is a structure-validation web service that evaluates model quality at both the global and local levels for proteins and nucleic acids. The SWISS-MODEL Structure Assessment page runs MolProbity version 4.4 as available from https://github.com/rlabduke/MolProbity. The Structure Assessment page hopes to show the most relevant scores provided by Molprobity and to identify where residues of low-quality lie in their model or structure. A table (2) of results is presented Molprobity values. For scores-per-residue (or residue-pair), the residues are sorted in decreasing order of quality so that the lowest quality residue (or residue-pair) is presented first. A tooltip provides the score for the residue/pair. The structure with less QMEAN values was chosen for further study.

2.2 Protein-Protein Interaction

The protein-protein interaction between our target proteins Cry1Ac, Cry1F, and Cry2Ab against our ligand proteins Cadherin and Cadherin-like proteins from *Helicoverpa*, *Pectinophora*, and *Spodoptera* was carried out by submitting their structural coordinates along with the active site residues predicted using CASTp to the HADDOCK server. HADDOCK (High Ambiguity Driven biomolecular Docking) is an information-driven flexible docking approach for the modelling of bio molecular complexes (Dominguez *et al.* 2003). HADDOCK (https://www.bonvinlab.org/software/haddock2.4/) is a collection of python scripts derived from ARIA (https://aria.pasteur.fr) that harness the power of CNS (Crystallography and NMR System – https://cns-online.org) for structure calculation of molecular complexes. The docking protocol of HADDOCK was designed so that the molecules experience varying degrees of flexibility and different chemical environments, and it can be divided into three different stages, each with a defined goal and characteristics: 1. Randomization of orientations and rigid-body minimization (it0), 2. Semi-flexible simulated annealing in torsion angle space (it1) and 3. Refinement in Cartesian space with explicit solvent (water). A result page showing the cluster statistics and some graphical representation of the data is obtained once the docking is successfully finished. The ranking of the clusters is based on the average score of the top 4 members of each cluster. The score is calculated as:

HADDOCKscore = 1.0 * Evdw + 0.2 * Eelec + 1.0 * Edesol + 0.1 * Eair

Evdw is the intermolecular van der Waals energy, Elec the intermolecular electrostatic energy, Edesol represents an empirical desolvation energy term adapted from Fernandez-Recio *et al.* . 2004, and Eair is the AIR energy. The cluster numbering reflects the size of the cluster, with cluster 1 being the most populated cluster. The various components of the HADDOCK score are also reported for each cluster on the results web page. Results are also obtained as graphical representations showing the distribution of the solutions for various measures (HADDOCK score, van der Waals energy) as a function of the Fraction of Common Contact with- and RMSD from the best-generated model (the best scoring model) along with the top 4 best structural coordinates of the complex for each cluster. The interface region of the top structure from each cluster was then analyzed and visualized using Discovery Studio 2016.

3. RESULTS AND DISCUSSION:

3.1Protein Structure Modelling

The three-dimensional structure for Cry1F, Cry2Ab from *Bacillus thuringenesis*, cadherin (CAD) and cadherin-like protein (BtR) from *Helicoverpa*, *Pectinophora*, and *Spodoptera* were determined using the homology modelling method in the Swiss-Model server workspace. The results are shown as a

superimposed image of the target and template as a cartoon ribbon model where the target is coloured in pink and the template in blue. The sequence alignment between the target and the template is shown along with a graphical representation of the QMEAN score observed for local and global structures. The Ramachandran plots for all the modelled structures are shown alongside their structures (Fig 1-8). The results are summarized in table 1 and table 2 for all the protein structures that were modelled and validated in Swiss-Model for the study.

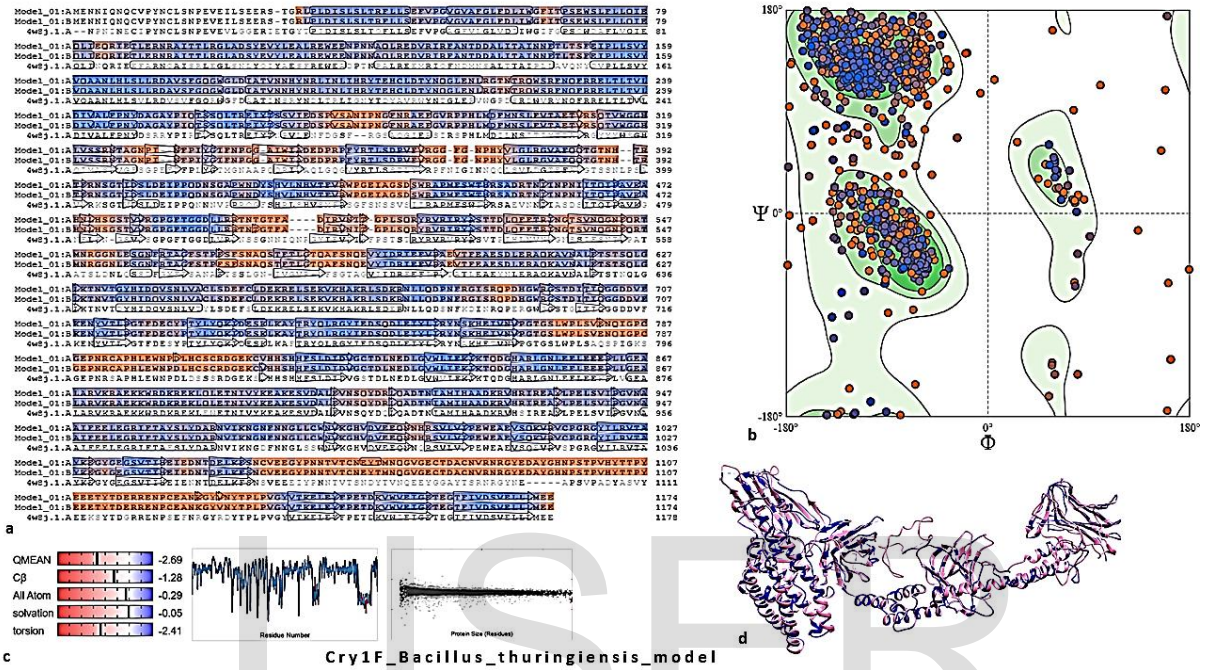


Fig 1 – Protein structure modeling of Cry1F from *Bacillus thuringiensis*. a - Sequence alignment, b – Ramachandran plot, c – Graphical representation of QMEAN Score, and d – Superimposed cartoon ribbon representation of Target (Pink) and Template (Blue).

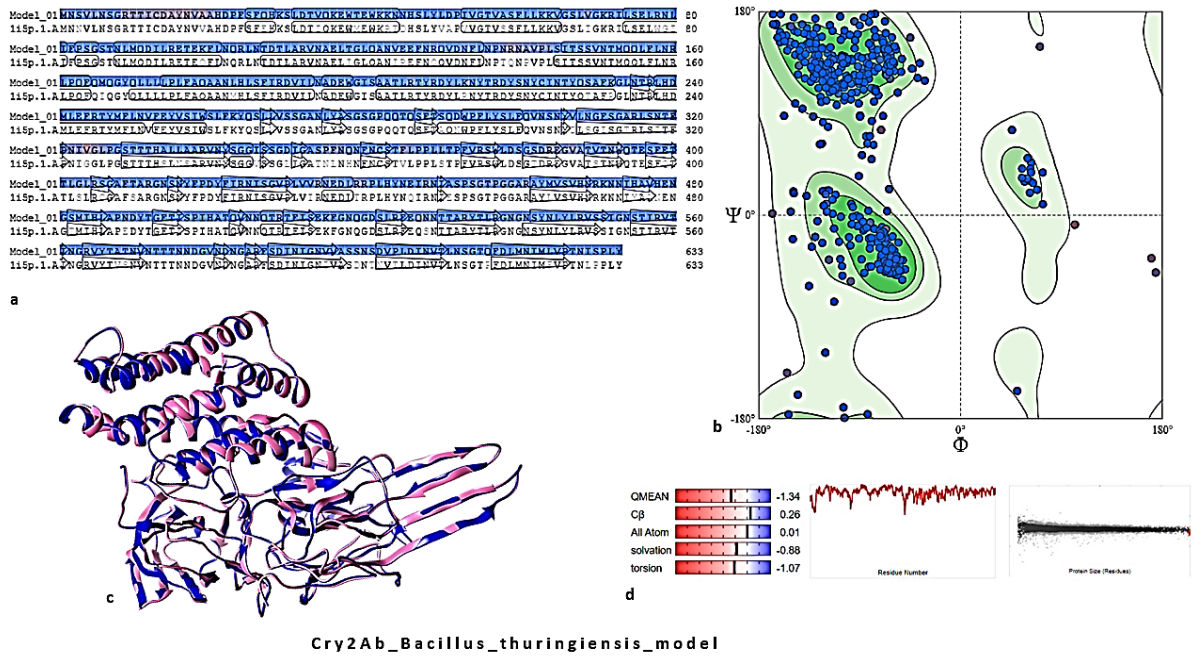


Fig 2 – Protein structure modeling of Cry2Ab from *Bacillus thuringiensis*. a - Sequence alignment, b – Ramachandran plot, c – Superimposed cartoon ribbon representation of Target (Pink) and Template (Blue), and d – Graphical representation of QMEAN Score.

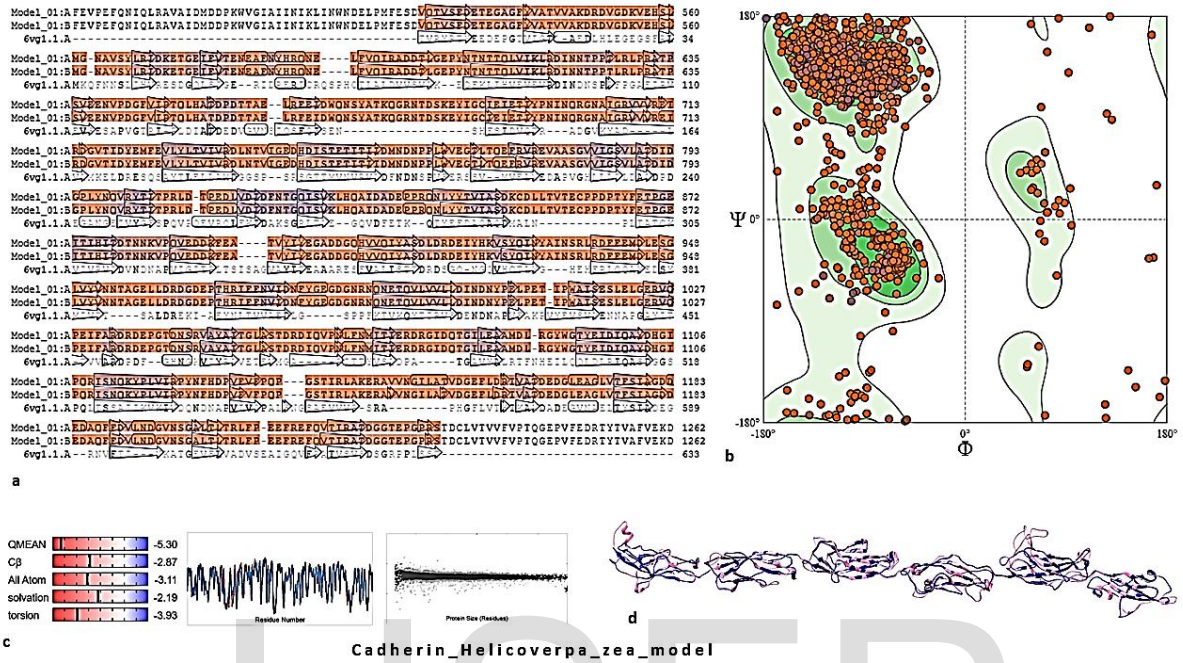


Fig 3 – Protein structure modeling of Cadherin (CAD) from *Helicoverpa zea*. a - Sequence alignment, b – Ramachandran plot, c – Graphical representation of QMEAN Score, and d – Superimposed cartoon ribbon representation of Target (Pink) and Template (Blue).

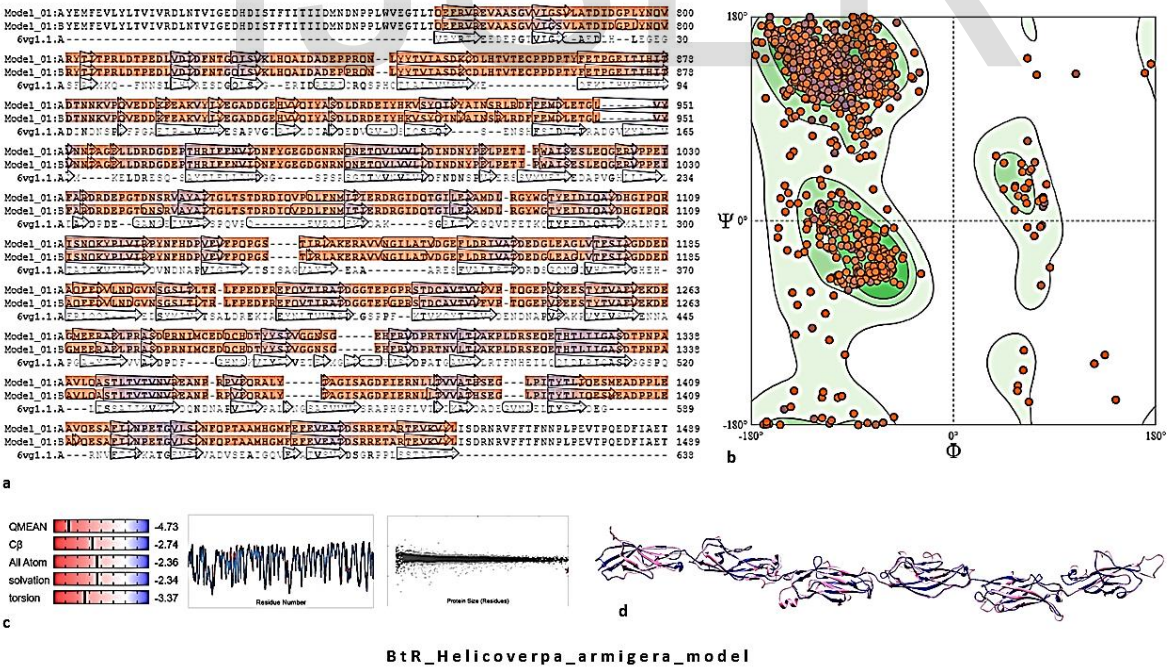


Fig 4 – Protein structure modeling of Cadherin-like protein (BtR) from *Helicoverpa armigera*. a - Sequence alignment, b – Ramachandran plot, c – Graphical representation of QMEAN Score, and d – Superimposed cartoon ribbon representation of Target (Pink) and Template (Blue).

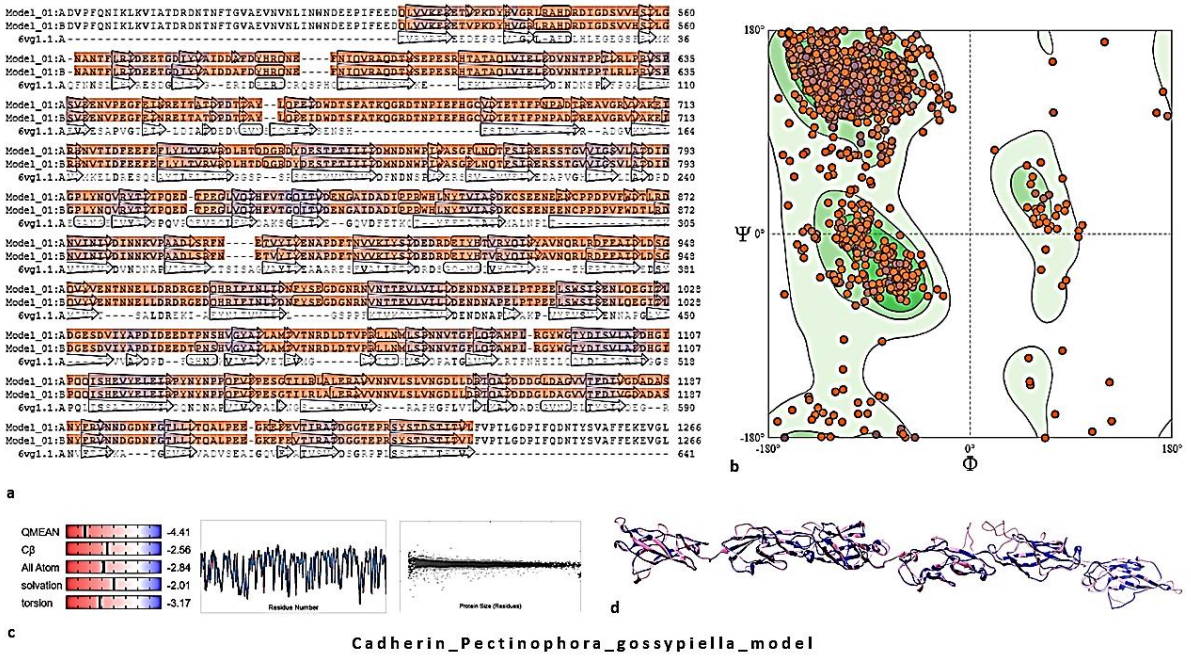


Fig 5 – Protein structure modeling of Cadherin (CAD) from *Pectinophora gossypiella*. a - Sequence alignment, b – Ramachandran plot, c – Graphical representation of QMEAN Score, and d – Superimposed cartoon ribbon representation of Target (Pink) and Template (Blue).

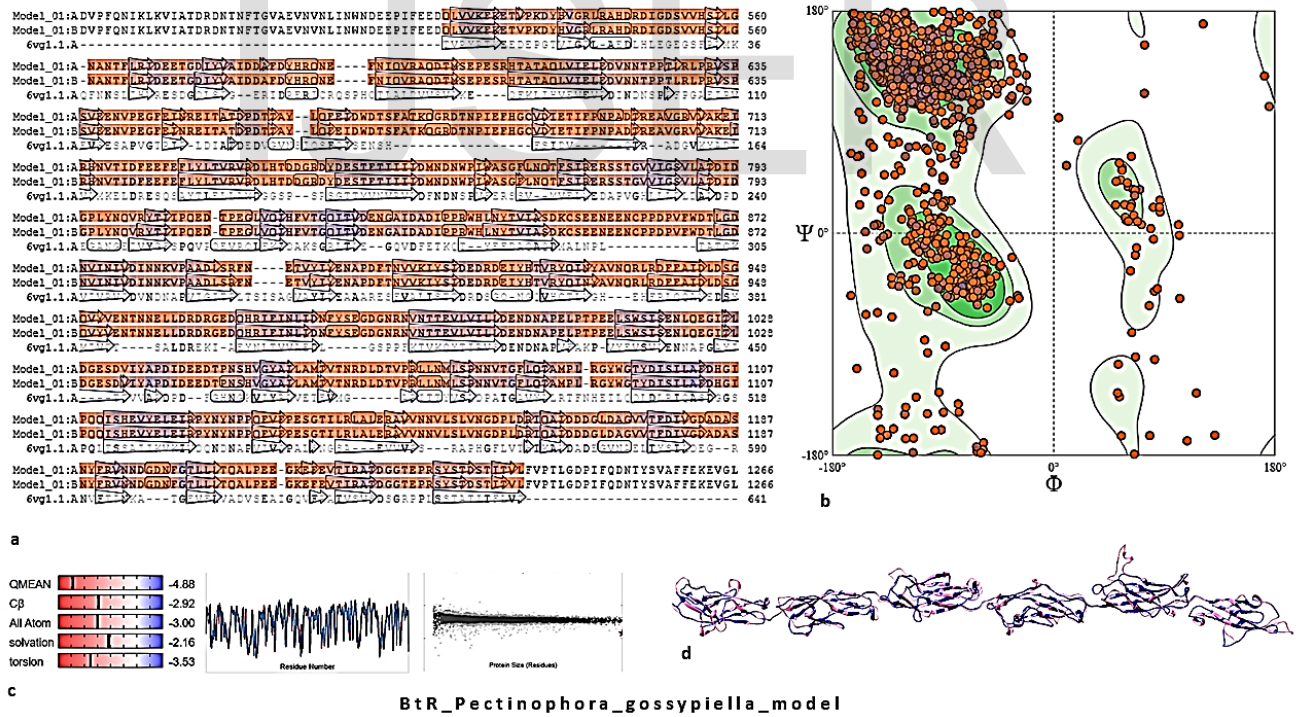


Fig 6 – Protein structure modeling of Cadherin-like protein (BtR) from *Pectinophora gossypiella*. a - Sequence alignment, b – Ramachandran plot, c – Graphical representation of QMEAN Score, and d – Superimposed cartoon ribbon representation of Target (Pink) and Template (Blue).

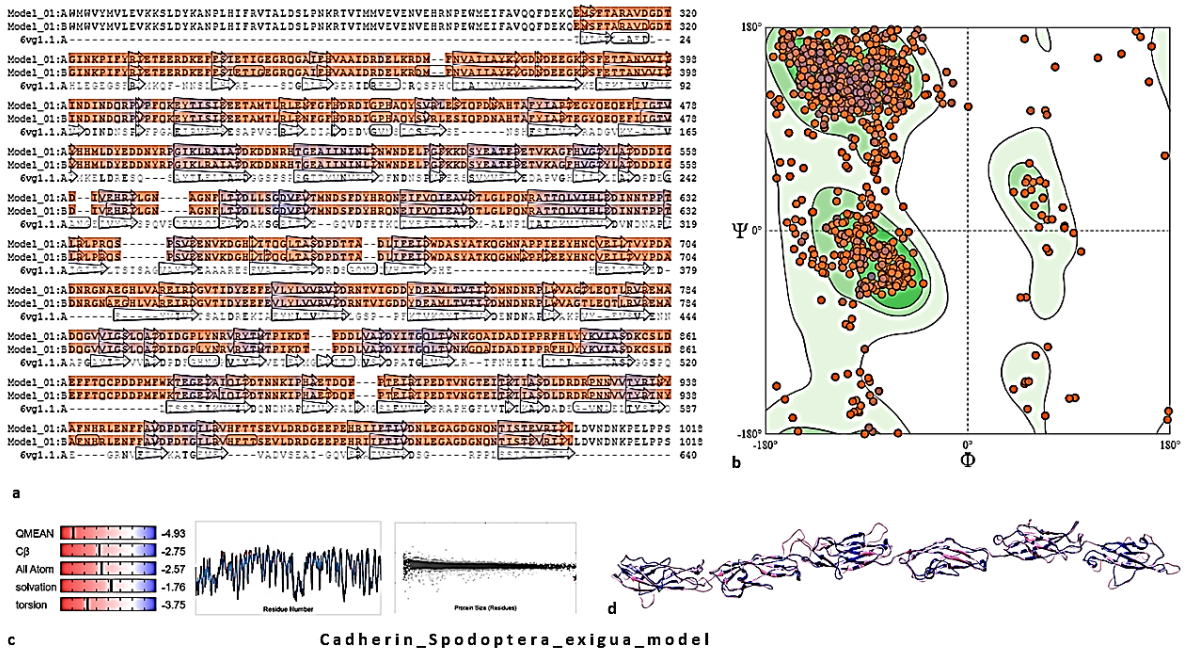


Fig 7 – Protein structure modeling of Cadherin (CAD) from *Spodoptera exigua*. a - Sequence alignment, b – Ramachandran plot, c – Graphical representation of QMEAN Score, and d – Superimposed cartoon ribbon representation of Target (Pink) and Template (Blue).

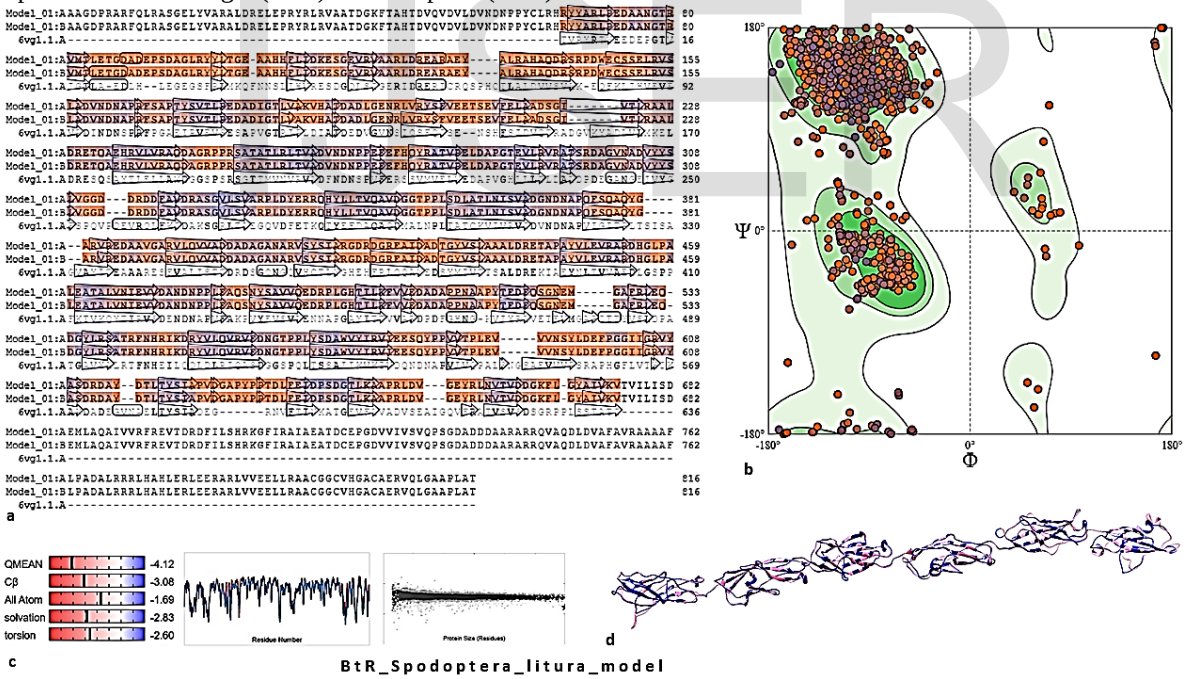


Fig 8 – Protein structure modeling of Cadherin-like protein (BtR) from *Spodoptera litura*. a - Sequence alignment, b – Ramachandran plot, c – Graphical representation of QMEAN Score, and d – Superimposed cartoon ribbon representation of Target (Pink) and Template (Blue).

Table 1 – Summary of the protein data involved in the modeling of the target proteins

Protein Model	UniProt ID	Template	Oligo-State	Range/Coverage	Sequence Identity

Cry1F (*Bacillus thuringiensis*)	B2ZPN5	Pesticidal Crystal Protein Cry1Ac (4W8J)	Homo-dimer	31-114/0.99	64.46
Cry2Ab (*Bacillus thuringiensis*)	P21254	Pesticidal Crystal Protein Cry2AA (1I5P)	Monomer	1-633/1.00	87.84
Cadherin (*Helicoverpa zea*)	A0A678NBJ2	Protocadherin Protein (6VG1)	Homo-dimer	527-1231/0.35	19.87
Cadherin-like protein (*Helicoverpa armigera*)	Q19KJ3	Protocadherin Protein (6VG1)	Homo-dimer	770-1461/0.35	21.65
Cadherin (*Pectinophora gossypiella*)	A0A060BFA9	Protocadherin Protein (6VG1)	Homo-dimer	525-1240/0.36	21.25
Cadherin-like protein (*Pectinophora gossypiella*)	Q86DU3	Protocadherin Protein (6VG1)	Homo-dimer	525-1240/0.36	21.25
Cadherin (*Spodoptera exigua*)	S5VXI8	Protocadherin Protein (6VG1)	Homo-dimer	308-1005/0.35	19.37
Cadherin-like protein (*Spodoptera litura*)	A0A0F6PPD3	Protocadherin Protein (6VG1)	Homo-dimer	66-675/0.73	26.97

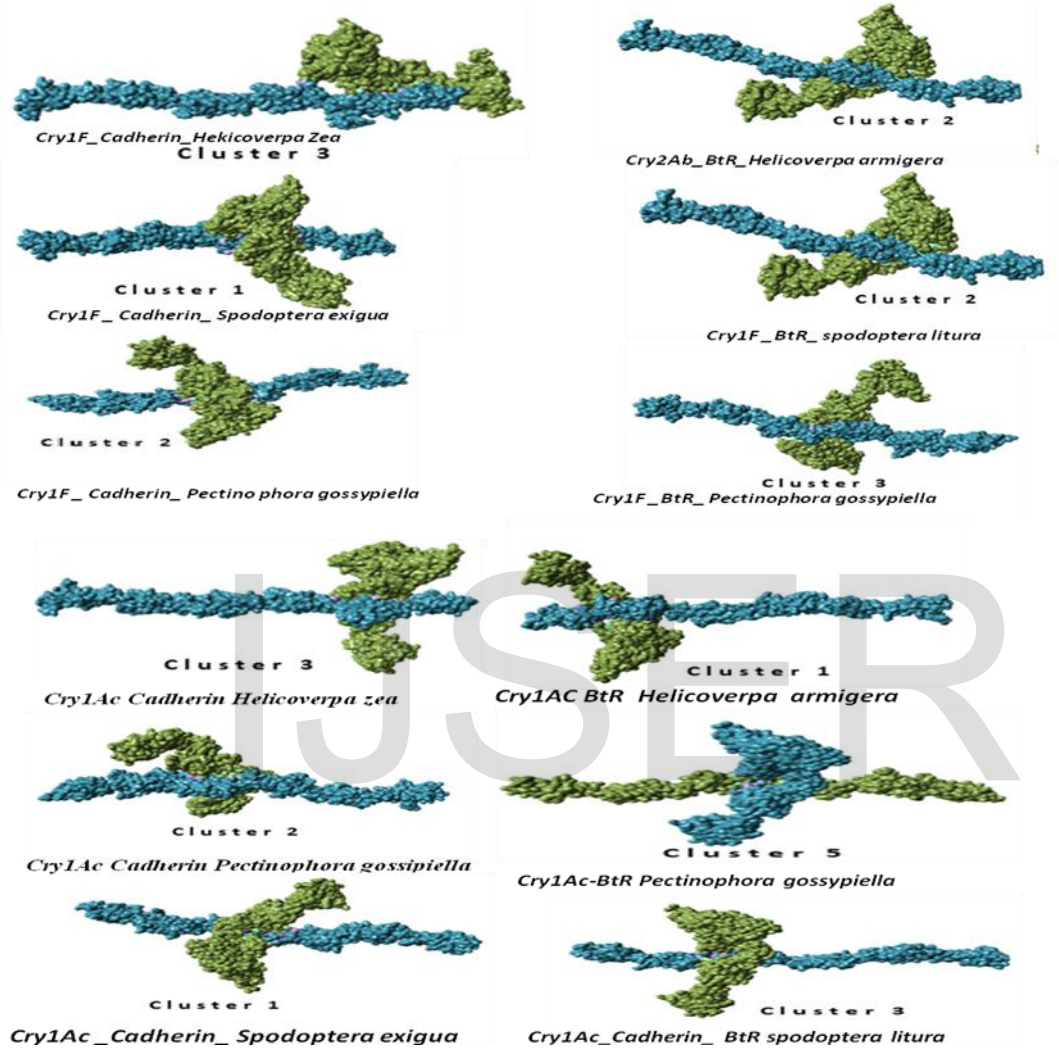
Table 2 – Summary of Validation of the protein structural data modelled from Swiss-Model

Protein Model	**MolProbity Score**	**Ramachandran Favoured**	**QSQE**	**GMQE**	**QMEAN**
Cry1F(*Bacillus thuringiensis*)	2.05	91.81%	0.42	0.7	-2.69
Cry2Ab(*Bacillus thuringiensis*)	1.84	93.03%	0	0.94	-1.34
Cadherin (*Helicoverpa zea*)	2.01	87.84%	0.16	0.15	-5.3
Cadherin-like protein (*Helicoverpa armigera*)	2.11	89.35%	0.14	0.15	-4.73
Cadherin (*Pectinophora gossypiella*)	1.91	87.54%	0.13	0.14	-4.41
Cadherin-like protein (*Pectinophora gossypiella*)	2	86.27%	0.13	0.14	-4.88
Cadherin (*Spodoptera exigua*)	1.97	86.06%	0.13	0.15	-4.93
Cadherin-like protein (*Spodoptera litura*)	1.57	91.45%	0.27	0.39	-4.12

3.2 Protein-Protein Interaction

The protein-protein interaction analysis for the bacterial protein against the insect cadherins was carried using macromolecular docking. For the study three bacterial proteins, Cry genes from *Bacillus thuringiensis (Cry1Ac*, *Cry1F*, and *Cry2Ab*) and six cadherins and cadherin-like proteins from the insect

species *Helicoverpa sp.*, *Pectinophora sp. and Spodoptera sp.* were chosen. The structure of these 8 proteins was predicted using homology modeling whereas for one protein (Cry1Ac) the NMR structure was present in the Protein Data Bank and was used for the analysis. The bacterial proteins were used as targets whereas the lepidopteran proteins were used as ligands for the macromolecular docking using the program HADDOCK. The active site regions were predicted for all 9 proteins using the CASTp server .



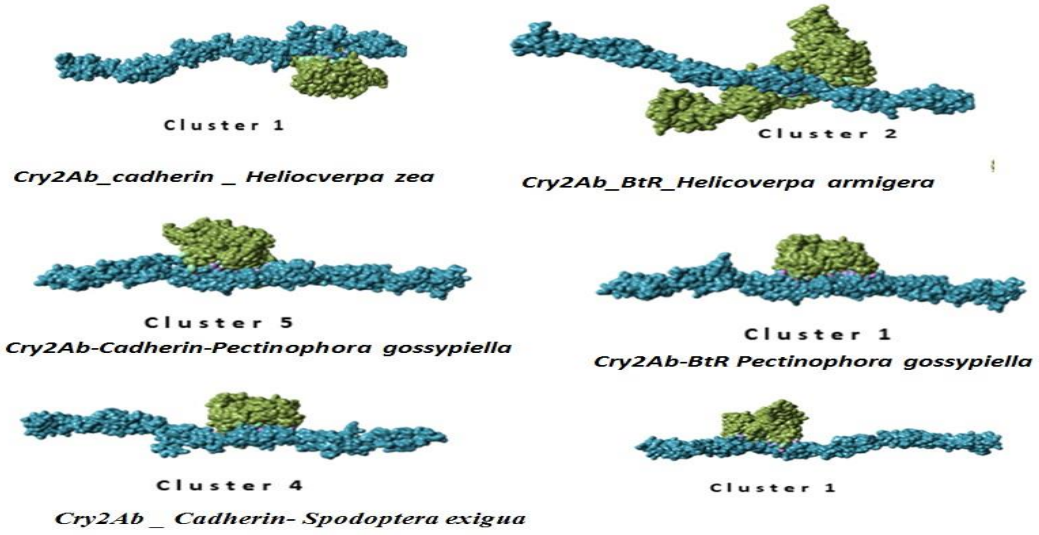
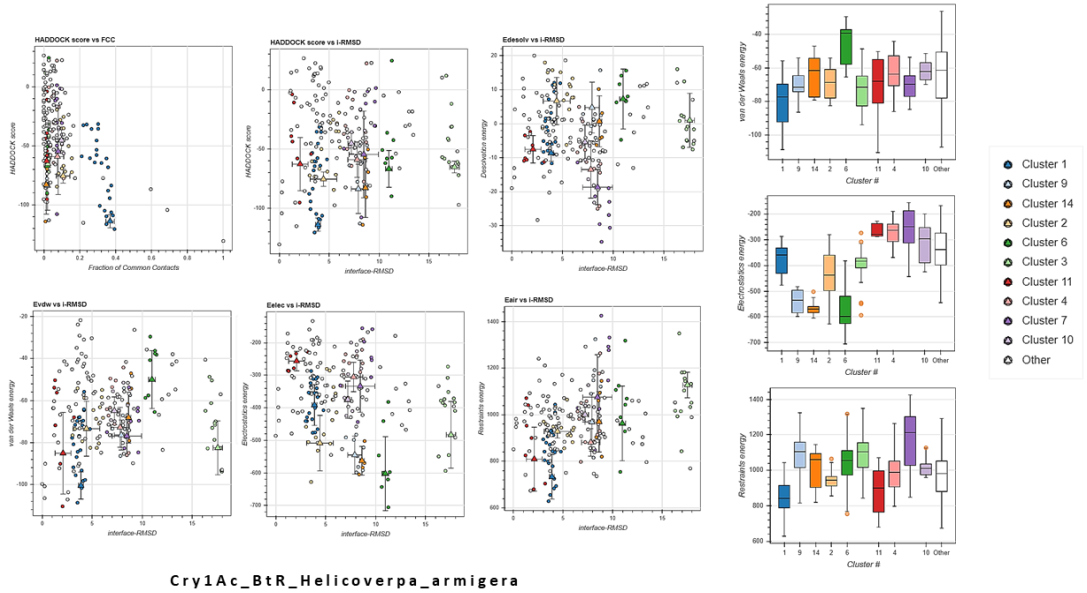


Fig 6: Protein-protein docking images using Discovery studio

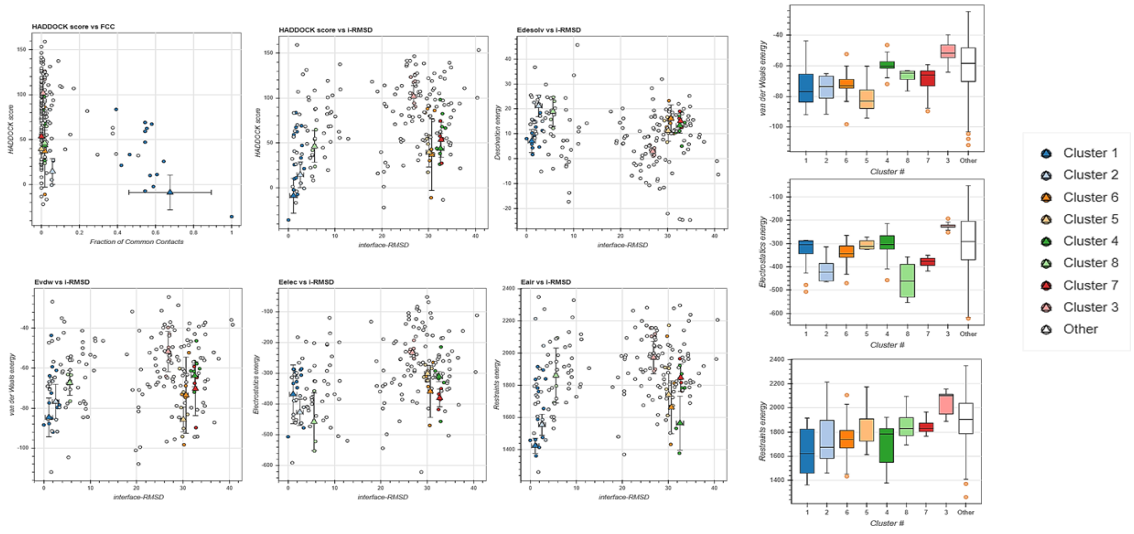
Then, the proteins were submitted to the HADDOCK server separately for each interaction between the target and ligand-protein. The results are obtained as the graphical representation (Graph.1) of various scores observed for the cluster groups. The results are summarized in the table (3, 4 and 5) for each target protein against the six ligand proteins.

4. Discussion:

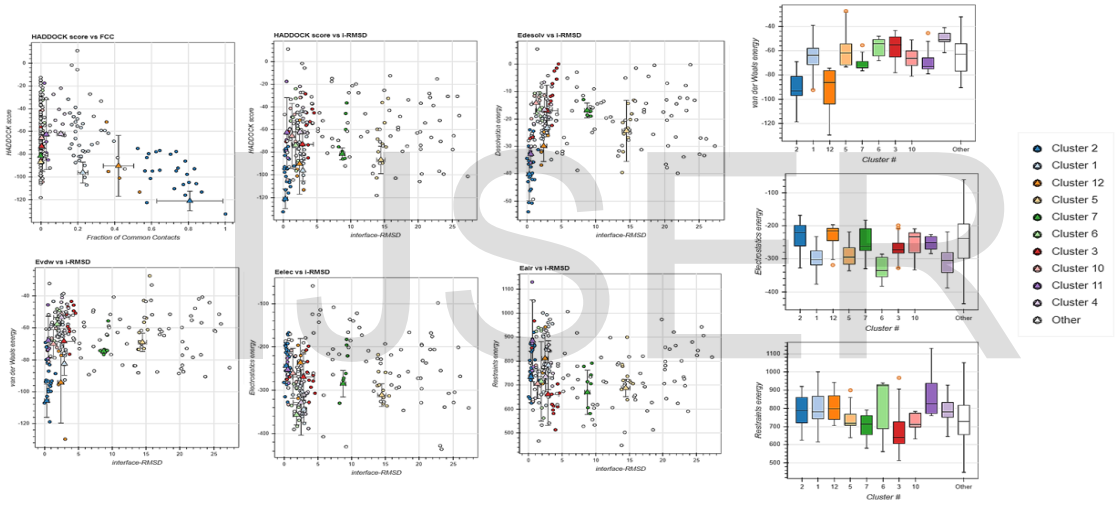
Based on the HADDOCK score and Z-Score, and the cluster with the best structure was determined. The lower the score, the best possible structure can be obtained. The best structure from each cluster was then analysed for the interacting residues in the interface region. The results revealed the highly active residues in the Cry genes. In Cry1Ac, the residues 548, 549, 783, 657, 822, 825, 923, 841, 654, 845, 580, 781, 666, 860 are highly active and formed interaction with all six insect cadherins. In Cry1F, the residues 851, 657, 870, 560, 808, 546, 611, 664, 656, 614, 629, 713, 772, 623, 621 and the residues 36, 63, 71, 490, 209, 29, 33, 30 in Cry2Ab are highly active forming salt bridges with the ligand-protein. The non-bond interaction such as electrostatics, conventional hydrogen bonds, hydrophobic interactions such as pi-alkyl stacking observed during the analysis of the interface regions is elaborated in the table.6 for the best complex in the cluster of each PPI with their distance radius expressed in Å.



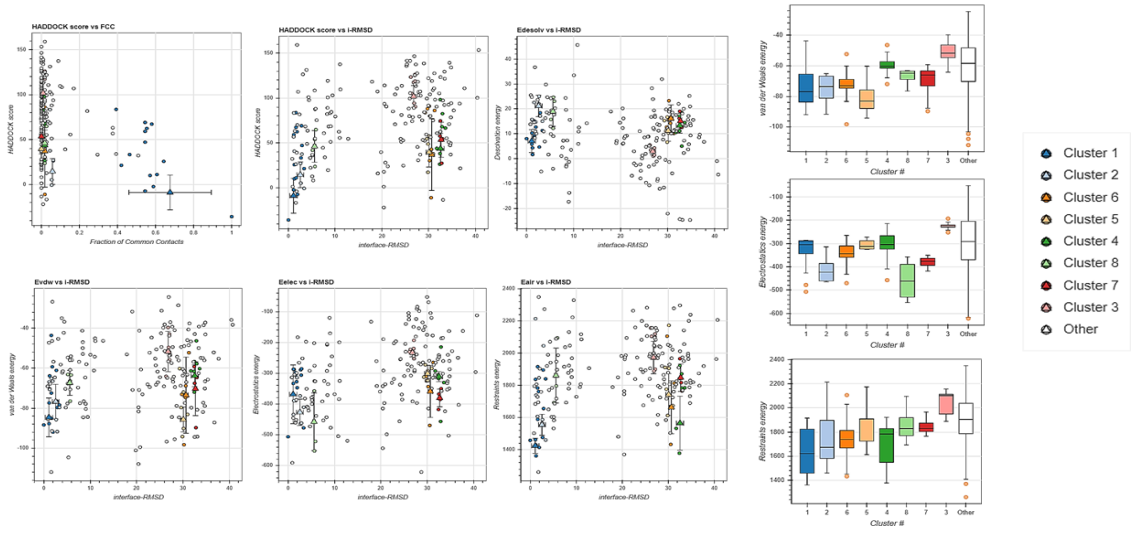
Cry1Ac_BtR_Helicoverpa_armigera



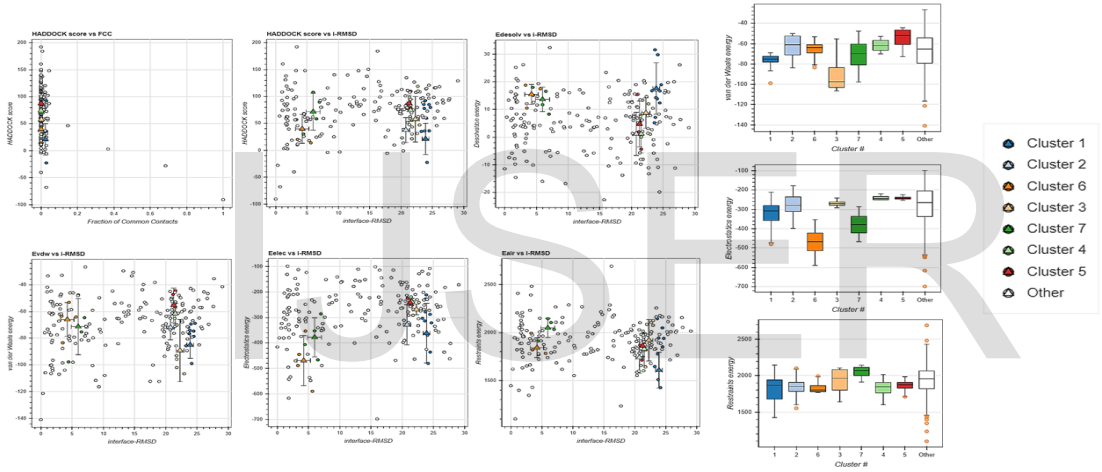
Cry1F_BtR_Spodoptera_litura



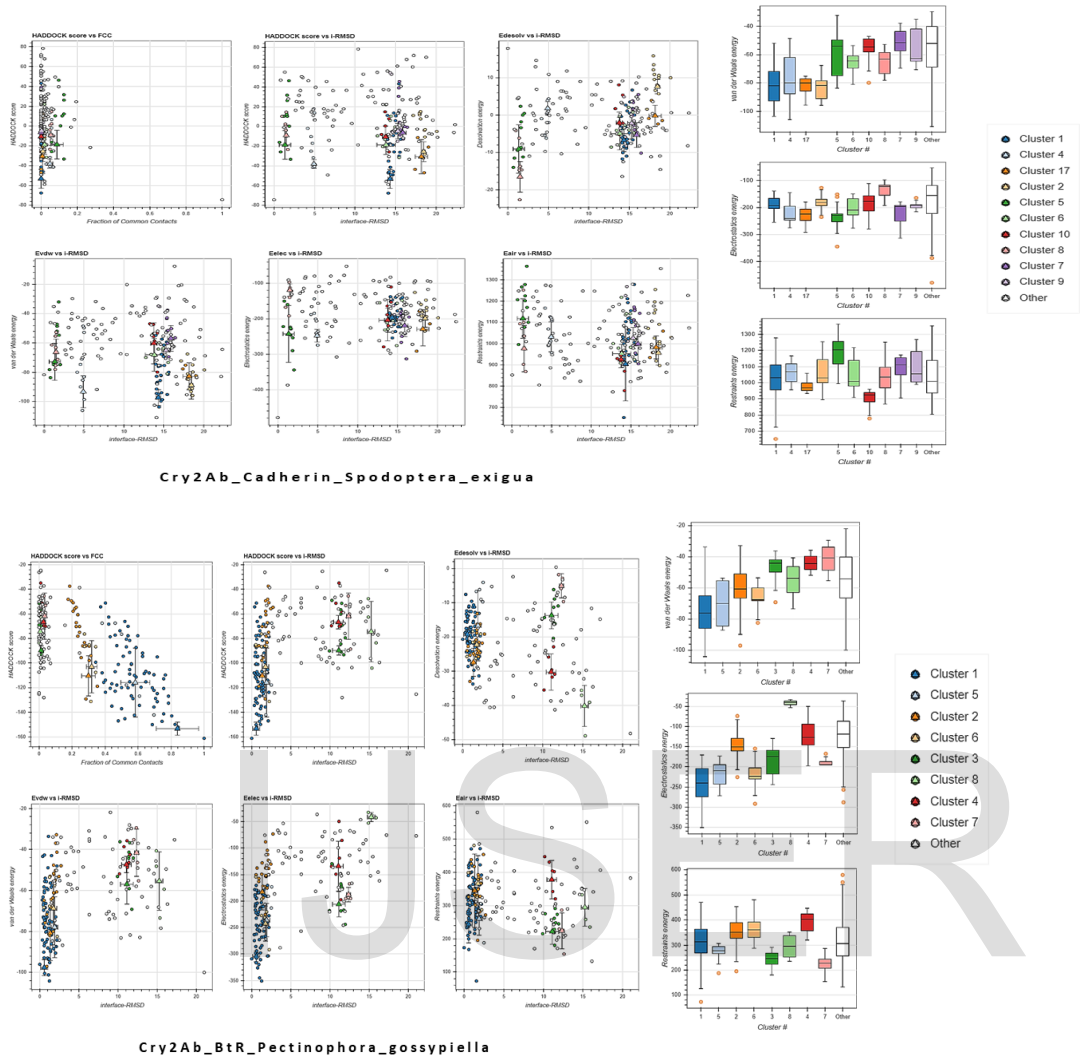
Cry1Ac_Cadherin_Pectinophora_gossypiella



Cry1F_BtR_Spodoptera_litura



Cry1F_Cadherin_Helicoverpa_zea



Graph 1: Protein –Protein docking interaction various scores with cluter group's representation

Table 3- HADDOCK Result obtained for cadherin proteins from Lepidoptera family against Cry1Ac of *Bacillus thuringiensis.*

Cry1Ac	Helicoverpa sp.		Pectinophora sp.		Spodoptera sp.	
	BtR	Cadherin	BtR	Cadherin	BtR	Cadherin
Cluster #	Cluster 1	Cluster 3	Cluster 3	Cluster 2	Cluster 2	Cluster 1
HADDOCK score	-114.0 +/- 4.8	-104.0 +/- 10.6	-160.0 +/- 10.9	-121.2 +/- 7.5	-129.3 +/- 8.9	-58.2 +/- 14.0
Cluster size	24	16	13	24	50	34
RMSD from the overall lowest-energy structure	10.2 +/- 0.3	19.7 +/- 0.5	33.8 +/- 0.0	1.4 +/- 1.6	0.5 +/- 0.3	17.1 +/- 0.7
Van der Waals energy	-100.5 +/- 5.6	-102.9 +/- 6.3	-104.9 +/- 8.9	-105.7 +/- 9.0	-94.8 +/- 11.0	-77.4 +/- 10.9
Electrostatic energy	-391.3 +/- 54.2	-317.2 +/- 34.9	-445.5 +/- 22.3	-243.8 +/- 35.8	-545.6 +/- 65.0	-347.6 +/- 25.1
Desolvation energy	-8.6 +/- 2.1	-1.2 +/- 2.5	-17.6 +/- 9.3	-40.2 +/- 8.4	14.9 +/- 4.1	4.9 +/- 0.9
Restraints	733.8 +/- 82.8	635.6 +/- 83.2	515.0 +/- 90.8	734.3 +/- 93.2	596.6 +/- 54.2	838.1 +/-

violation energy						118.2
Buried Surface Area	3466.6 +/- 67.4	3321.8 +/- 126.5	3539.0 +/- 142.7	3425.3 +/- 80.7	3587.7 +/- 84.0	3013.0 +/- 140.2
Z-Score	-2.3	-2.1	-2.1	-2.3	-2.1	-1.5

Table 4- HADDOCK Result obtained for cadherin proteins from lepidoptera family against Cry1F of Bacillus thuringiensis.

Cry1F	**Helicoverpa sp.**		**Pectinophora sp.**		**Spodoptera sp.**	
	BtR	**Cadherin**	**BtR**	**Cadherin**	**BtR**	**Cadherin**
Cluster #	Cluster 2	Cluster 1	Cluster 5	Cluster 2	Cluster 1	Cluster 3
HADDOCK score	-8.4 +/- 16.9	21.1 +/- 25.2	-58.9 +/- 32.1	-57.8 +/- 8.5	-8.8 +/- 16.8	-47.2 +/- 10.3
Cluster size	10	10	6	10	14	13
RMSD from the overall lowest-energy structure	20.0 +/- 0.3	43.7 +/- 1.0	2.6 +/- 1.7	8.9 +/- 0.3	1.4 +/- 0.9	1.2 +/- 1.0
Van der Waals energy	-89.3 +/- 6.4	-85.0 +/- 8.7	-96.4 +/- 27.7	-87.9 +/- 7.6	-84.6 +/- 8.4	-90.1 +/- 15.7
Electrostatic energy	-565.2 +/- 96.3	-361.8 +/- 101.2	-551.0 +/- 53.3	-649.0 +/- 20.7	-368.7 +/- 82.6	-676.9 +/- 33.6
Desolvation energy	12.0 +/- 2.3	17.6 +/- 8.1	0.1 +/- 4.0	4.2 +/- 2.8	7.1 +/- 4.0	21.0 +/- 3.2
Restraints violation energy	1820.2 +/- 96.5	1608.4 +/- 161.0	1475.9 +/- 90.1	1557.3 +/- 152.4	1424.6 +/- 40.9	1572.8 +/- 134.8
Buried Surface Area	3850.6 +/- 128.9	3172.0 +/- 376.7	3775.0 +/- 325.6	3843.3 +/- 150.3	3532.6 +/- 166.6	3912.2 +/- 427.4
Z-Score	-2.3	-1.6	-1.8	-1.8	-1.7	-2.5

Table 5- HADDOCK Result obtained for cadherin proteins from Lepidoptera family against Cry2Ab of *Bacillus thuringiensis.*

Cry2Ab	**Helicoverpa sp.**		**Pectinophora sp.**		**Spodoptera sp.**	
	BtR	**Cadherin**	**BtR**	**Cadherin**	**BtR**	**Cadherin**
Cluster #	Cluster 1	Cluster 1	Cluster 1	Cluster 5	Cluster 4	Cluster 1
HADDOCK score	-58.7 +/- 2.4	-89.6 +/- 6.1	-153.4 +/- 4.6	-120.0 +/- 10.4	-93.9 +/- 22.9	-53.1 +/- 8.4
Cluster size	41	70	74	16	19	27
RMSD from the overall lowest-energy structure	15.0 +/- 0.5	2.6 +/- 1.8	0.7 +/- 0.4	31.2 +/- 0.0	1.9 +/- 1.4	30.5 +/- 0.1
Van der Waals energy	-68.7 +/- 8.5	-80.9 +/- 8.2	-97.6 +/- 3.4	-102.2 +/- 12.8	-97.9 +/- 13.4	-97.3 +/- 4.7
Electrostatic energy	-356.0 +/- 53.0	-271.1 +/- 12.5	-304.4 +/- 8.8	-158.3 +/- 23.5	-246.0 +/- 52.1	-197.0 +/- 6.0
Desolvation energy	-1.0 +/- 2.3	-1.3 +/- 5.3	-19.2 +/- 1.2	-34.4 +/- 4.0	-0.0 +/- 3.3	-6.7 +/- 2.6
Restraints violation energy	821.1 +/- 83.2	468.5 +/- 11.5	243.8 +/- 48.8	482.1 +/- 35.9	532.0 +/- 54.2	903.7 +/- 149.8
Buried Surface Area	2627.3 +/- 123.0	3009.5 +/- 215.1	3160.5 +/- 64.3	3220.5 +/- 211.3	3123.7 +/- 331.6	3037.2 +/- 64.5
Z-Score	-1.1	-1.5	-2	-1.3	-1.8	-2.1

Table 6: Analysis of Protein –protein interactions

From	**To**	**Types**	**Distance Å**
B:ARG707:HH12	A:ASP713:OD1	Salt Bridge: Attractive Charge	1.58
B:ARG707:HH22	A:ASP713:OD2	Salt Bridge; Attractive Charge	1.623

A:SER549:HN	B:ARG629:O	Conventional H-Bond	1.868
A:ARG666:HE	B:ASP853:OD2	Conventional H-Bond	1.745
Helicoverpa-BtR			
A:LYS665:HZ2	B:GLU906:OE1	Salt Bridge; Attractive Charge	1.734
A:LYS717:HZ2	B:GLU946:O	Conventional H-Bond	1.592
B:TYR899:HH	A:GLU659:O	Conventional H-Bond	1.768
B:ARG918:HE	A:GLY780:O	Conventional H-Bond	1.703
B:LYS924:HZ3	A:GLY782:O	Conventional H-Bond	1.872
Pectinophora-CAD			
A:ARG666:HH11	B:GLU982:OE2	Salt Bridge; Attractive Charge	2.644
A:ARG666:HH12	B:GLU982:OE1	Salt Bridge;Attractive Charge	1.643
A:ARG511:HH22	B:PRO864:O	Conventional H-Bond	1.82
A:SER548:HG	B:THR845:OG1	Conventional H-Bond	1.704
Pectinophora-BtR			
B:ARG511:HH12	A:GLU916:OE2	Salt Bridge;Attractive Charge	2.389
B:ARG511:HH22	A:GLU916:OE2	Salt Bridge;Attractive Charge	1.613
B:THR554:HG1	A:ASP833:OD2	Conventional H-Bond	1.802
B:LYS623:HZ3	A:ASP809:O	Conventional H-Bond	1.753
Spodoptera-CAD			
B:ARG594:HH11	A:ASP758:OD2	Salt Bridge;Attractive Charge	1.665
B:ARG594:HH12	A:GLU757:OE2	Salt Bridge;Attractive Charge	1.583
A:HIS825:HE2	B:GLU711:OE2	Conventional H-Bond	1.63
B:LYS530:HZ2	A:SER548:OG	Conventional H-Bond	1.641
Spodoptera-BtR			
A:ARG526:HH12	B:ASP313:OD2	Salt Bridge; Attractive Charge	1.619
A:ARG526:HH21	B:ASP313:OD1	Salt Bridge; Attractive Charge	1.643
B:ARG297:HH22	A:GLU757:OE2	Salt Bridge; Attractive Charge	1.631
Cry1F-Helicoverpa-CAD			
A:ARG870:HH12	B:GLU583:OE1	Salt Bridge; Attractive Charge	1.793
A:ARG870:HH22	B:GLU583:OE1	Salt Bridge; Attractive Charge	1.705
A:ILE184:HN	B:ASP758:OD1	Conventional H-Bond	1.772
A:ASN192:HD22	B:ASP643:OD1	Conventional H-Bond	1.679
A:ARG200:HH12	B:ARG714:O	Conventional H-Bond	1.689
Helicoverpa-BtR			
A:LYS664:HZ3	B:GLU906:OE2	Salt Bridge; Attractive	3.15

		Charge	
A:ARG851:HH22	B:GLU920:OE2	Salt Bridge; Attractive Charge	1.657
B:LYS924:HZ3	A:ASP705:OD2	Salt Bridge; Attractive Charge	1.575
A:LYS708:HZ1	B:THR947:O	Conventional H-Bond	1.899
A:ILE784:HN	B:GLU888:OE2	Conventional H-Bond	1.938
Spodoptera-CAD			
A:ARG808:HH11	B:GLU524:OE2	Salt Bridge;Attractive Charge	1.818
A:ARG808:HH22	B:GLU524:OE2	Salt Bridge;Attractive Charge	2.39
A:ASN586:HD21	B:ASP531:OD1	Conventional H-Bond	2.042
Spodoptera-BtR			
A:LYS614:HZ1	B:GLU177:OE1	Salt Bridge;Attractive Charge	1.612
A:LYS614:HZ2	B:GLU231:OE1	Salt Bridge;Attractive Charge	1.731
A:LYS614:HZ3	B:ASP265:OD2	Salt Bridge;Attractive Charge	1.597
B:GLN346:HE22	A:ASP527:OD1	Conventional H-Bond	1.731
B:ASN362:HD22	A:ASP809:OD2	Conventional H-Bond	1.732
Pectinophora-CAD			
A:ARG546:HH21	B:ASP984:OD1	Salt Bridge;Attractive Charge	3.054
A:ARG546:HH22	B:ASP919:OD2	Salt Bridge;Attractive Charge	1.577
A:ARG560:HE	B:GLU920:OE1	Conventional H-Bond	2.093
A:LYS614:HZ3	B:LEU945:O	Conventional H-Bond	1.657
B:THR845:HG1	A:ARG808:O	Conventional H-Bond	1.782
B:LEU945:HN	A:GLU610:OE1	Conventional H-Bond	1.946
Pectinophora-BtR			
A:LYS614:HZ1	B:ASP946:OD2	Salt Bridge;Attractive Charge	1.553
A:ARG808:HH12	B:ASP872:OD2	Salt Bridge;Attractive Charge	1.529
A:LYS812:HZ1	B:ASP809:OD1	Salt Bridge;Attractive Charge	1.58
A:SER622:HG	B:LEU889:O	Conventional H-Bond	1.836
B:LEU945:HN	A:GLU610:OE1	Conventional H-Bond	1.936
Cry2Ab-Helicoverpa-CAD			
B:ARG629:HH11	A:GLU76:OE2	Salt Bridge;Attractive Charge	1.554
B:ARG707:HH12	A:ASP490:OD1	Salt Bridge;Attractive Charge	1.573
B:ARG707:HH22	A:ASP490:OD1	Salt Bridge;Attractive Charge	2.278

B:GLN650:HE22	A:LYS63:O	Conventional H-Bond	1.983
B:HIS652:HD1	A:HIS28:O	Conventional H-Bond	2.688
B:THR654:HG1	A:SER30:OG	Conventional H-Bond	1.918
B:ARG701:HH22	A:THR401:O	Conventional H-Bond	1.718
Helicoverpa-BtR			
A:LYS36:HZ3	B:GLU920:OE2	Salt Bridge;Attractive Charge	1.78
B:ARG807:HH11	A:GLU126:OE2	Salt Bridge;Attractive Charge	1.511
B:ARG918:HH22	A:ASP32:OD1	Salt Bridge;Attractive Charge	1.852
A:ASN2:HD21	B:GLU906:OE2	Conventional H-Bond	1.855
B:ARG918:HE	A:ASP32:OD1	Conventional H-Bond	1.864
Spodoptera-CAD			
B:HIS713:HE2	A:ASP132:OD1	Salt Bridge;Attractive Charge	2.07
A:ASN489:HD21	B:ARG594:O	Conventional H-Bond	1.908
A:ARG537:HH21	B:GLY567:O	Conventional H-Bond	1.688
A:ASN600:HD21	B:PHE599:O	Conventional H-Bond	1.813
Spodoptera litura-BtR			
A:MET1:HN	B:ASP229:OD2	Salt Bridge;Attractive Charge	1.978
A:LYS64:HZ1	B:ASP313:OD2	Salt Bridge;Attractive Charge	1.642
A:ASN2:HD21	B:ASP265:OD1	Conventional H-Bond	1.645
A:ASN2:HD22	B:GLU231:OE1	Conventional H-Bond	1.97
Pectinophora gossypiella-CAD			
A:ARG209:HH12	B:ASP944:OD2	Salt Bridge;Attractive Charge	3.298
A:ARG209:HH21	B:ASP944:OD2	Salt Bridge;Attractive Charge	1.85
A:SER30:HN	B:ASN843:OD1	Conventional H-Bond	1.959
Pectinophora gossypiella -BtR			
A:ARG129:HH21	B:ASP904:OD2	Salt Bridge;Attractive Charge	1.612
B:ARG918:HH11	A:ASP22:OD2	Salt Bridge;Attractive Charge	1.611
A:ARG129:HH22	B:ASP904:O	Conventional H-Bond	1.831
A:ASN138:HD21	B:GLU953:OE2	Conventional H-Bond	1.919

In previous reports protein –protein docking results were included that the interaction residues Arg 368 and 369 of Cry1Ac were involved in the toxin protein with insect mid gut specifically Lepidopteran (Leelavathi *et al* 1999 & 2000).The residues including within the range of 503-525 (Avisar *et al*., 2004) were involved in interaction. Senguptha *et al* (2013) reported that the amino acid residues Q509, N510, R511, Y513 and W545 form binding site that can interact with insect proteins. Ser290, Ser293, Arg289, Glu332, Leu337, Gly339, Thr340 and Arg437 were novel residues involved in protein- protein interaction were reported by Ahamd *et al*. (2015).

In this study aminoacid residues of Cry1AcARG707, SER549, ARG666, GLY780, LYS665, LYS717, TYR899, ARG918, Cry1F aminoacid residues LYS664, HIS876, LYS924, LEU945, THR845, LYS614, ARG200, ASP527 and Cry2Ab GLN650, THR654, ARG701, ARG807, ASN2, ARG918 amino acid residues are involved in Protien-protein interactions represented in Table. 6. The lowest Z scores of Cry1Ac with *Helicoverpa* BtR (-2.3), *Pectinophora* Cadherin (-2.3), *Spodoptera* BtR (-2.1), Cry1F with *Helicoverpa* BtR (-2.3) and *Spodoptera* CAD (-2.5) Cry2Ab with *Pectinophora* BtR (-2) and *Spodoptera* CAD (-2.1) considered as best interacted models.

5. Conclusion:

In conclusion, it is represented here three target cry toxins and 6 insect cadherin and cadherin like proteins were chosen and modelled through the Swiss model workspace and do not share common 3-dimentional structure. The lowest HADdock scores and Z scores considered as best interacting molecules and determined as best closed structures. The interaction between target cry toxin proteins with Cadherin like proteins of *Helicoverpa* species showed the best models based on their Z-scores (-2.1,-2.3 and -2.5). These results useful for further elucidation of interactions between three cry toxins –insect receptor cadherin and cadherin like receptors and thereby increase the insecticidal protein's activity through molecular modification.

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Conflict of interest statement

Authors declare that there is no conflict of interest

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