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RESEARCH ARTICLE

In Silico Analysis of Antiviral Proteins in *Clerodendrum Inerme*

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ABSTRACT

Clerodendrum inerme is used in Indian and Chinese traditional medicine. It is reported to exhibit antiviral property against mammalian viruses. In this study, antiviral property of *C. inerme* against the economically important plant viruses such as, Cowpea mosaic virus (CoMV), Cowpea chlorotic mottle virus (CCMV), Cucumber mosaic virus (CuMV), Potato yellow mosaic virus (PYMV), Satellite tobacco mosaic virus (STMV) and Tobacco mosaic virus (TMV) is analyzed *in silico* using protein-protein interaction studies. The proteins in *C. inerme*, *viz*., CIP-29 and Crip-31 were found to exhibit antiviral property. Site directed mutagenesis of these two proteins using *in silico* methods was also studied. The analysis revealed that there is effective binding of the viral proteins and plant proteins with minimum binding energy. The study also revealed that the native plant proteins, CIP-29 and Crip-31are more effective to interact with viral proteins than the *in silico* mutated versions.

KEYWORDS

Clerodendrum inerme, Antiviral property, *In silico* analysis, Site directed mutagenesis, ClusPro Server 2.0

INTRODUCTION

Clerodendrum inerme Gaertn is cosmopolitan in distribution and has been used in Indian and Chinese traditional medicine for ages. In Indian tribal medicine, leaves of *Clerodendrum inerme* are used for treating fever, cough, skin rashes and boils, and are used in conjunction with other plant leaves.

It is used to treat umbilical cord infection and for cleaning the uterus (Rajasekaran *et al*, 2006). *Clerodendrum inerme* is also traditionally used as an abortifacient (Gurudeeban *et al*, 2010). In addition to this, antiviral activity of *Clerodendrum inerme* against Hepatitis B virus was also documented (Mehdi *et al*., 1997).

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Systemic antiviral resistance-inducing protein, CIP-29 was reported in *Clerodendrum inerme* Gaertn. It was found that CIP-29 has the characteristics of a polynucleotide adenosine glycosidase (ribosome-inactivating protein), which inhibits protein synthesis both in cell-free systems and, at higher concentrations, in cells, and releases adenine from ribosomes, RNA, poly (A) and DNA. As compared with other known RIPs, CIP-29 deadenylates DNA at a high rate, and induces systemic antiviral resistance. CIP-29 purified from *Clerodendrum inerme*, induced a very high degree of systemic resistance against tobacco mosaic virus in *Nicotiana tabacum* cv. Samsun NN (Fabiola *et al*, 1996).

A novel protein designated Crip-31 with properties similar to RIPs, isolated from *Clerodendrum inerme* was reported to induce

both localized and systemic resistance against cytomegalovirus (CMV), Potato virus Y (PVY) and tomato mosaic virus(ToMV) (Schelly et al., 2001).

Thus this study was framed with the following objective: To study the antiviral property of the proteins CIP-29 and Crip-31 of *Clerodendrum inerme* against economically important plant viruses by *in silico* analysis.

MATERIALS AND METHODS

The protein sequences of CIP-29 and Crip-31 were taken from NCBI.

Protein Modeling

Before modeling the protein the reference structures which acts as a template was selected by using Protein-BLAST (blastp) against PDB. Homology modeling was done using the following coding under the software MODELLER9.10.

Structure Evaluation and Validation

The structures generated by the modeller9.10 were validated using the SAVES (Structural Analysis and Verification Server) server by generating the Ramachandran plot. The Z- mean score of the model structures was estimated by using WHATIF server. Using Procheck and Ramachandran plot, the stereochemical quality of the protein structure was assessed.

The force field calculation was done to evaluate the energy of the structure and to repair disorted geometrics through energy minimization.

Protein-Protein Docking

Protein-Protein docking was done using ClusPro server 2.0. It is the first fully integrated server that includes both docking and discrimination steps for predicting the structure of protein–protein complexes. The server can be used to discriminate a set of potential complex structures from several docking algorithms, or it can generate its own structures using DOT or ZDOCK (Comeau *et al*, 2004). It gives the results as the docked structures. Based on the energy values of each model the structures were generated. The interaction studies was done for both the receptors with the ligands mentioned below.

Plant Proteins	Viral Proteins
➢ CIP-29 ➢ Crip-31	REPLICASE PROTEIN: ➢ Cowpea mosaic virus (CoMV) COAT PROTEIN: ➢ Cowpea chlorotic mottle virus (CCMV) ➢ Cucumber mosaic virus (CuMV) ➢ Potato yellow mosaic virus (PYMV) ➢ Satellite tobacco mosaic virus (STMV) ➢ Tobacco mosaic virus (TMV)

In ClusPro server, the structures based on the balanced type of interaction was studied, as it included electrostatic, hydrophobic, vanderwaals + electrostatic interactions which were preferred for docking.

Interaction Studies

Protein Interactions Calculator (PIC) is a server which recognizes various kinds of interactions such as disulphide bonds, hydrophobic interactions, ionic interactions, hydrogen bonds, aromatic-aromatic interactions, aromatic-sulphur interactions and cation $-\pi$ interactions within a protein or between proteins in a complex.

The amino acid residues involved in these interactions were retrieved. Interactions were calculated based on empirical or semi-empirical set of rules.

In Silico Site Directed Mutagenesis

Site directed mutagenesis is the mutation done to the active site residues of the receptor proteins. The mutation is done to study the significance of particular amino acid residues in the active site.

Site Directed Mutagenesis is done using the tool SwissPDBviewer (SPDBV_4.04).

In Silico Site Directed Mutagenesis of Cip-29

For CIP-29 three of six interactions with different ligands show that tyrosine residue in 271th position is common in all the interactions which were obtained by protein interactions calculator (PIC). And thus the 271th position which is tyrosine (aromatic hydrophobic amino acid) was mutated into tryptophan and phenylalanine (aromatic hydrophobic amino acids) and Isoleucine (aliphatic hydrophobic amino acid).

In Silico Site Directed Mutagenesis of Crip-31

For Crip-31 three of six interactions with different ligands show that proline residue in 188th position is common in all the interactions which were obtained by protein interactions calculator (PIC). And thus the 188th position which is proline (neutral, special case amino acid of hydrophobic amino acids) was mutated into phenylalanine (aromatic amino acid of hydrophobic amino acids) and cysteine (hydrophobic amino acid).

RESULTS

The protein sequence was retrieved from NCBI for both CIP-29 and Crip-31. These are the complete CDS sequences that were obtained experimentally and it is available for public use in NCBI. The accession number and the amino acid length of the sequences are given below:

Protein	Accession Number	Amino Acid Length
CIP-29	EU839992	912 bp
Crip-31	AF529299	941 bp

Blastp Results

The protein sequence of CIP-29 and Crip-31 was analysed in blastp program of NCBI. The PDB Blast was done to find the best template structure for modeling the proteins. Table below shows that the structure of 2QES is the best template for both the proteins for homology modeling.

Protein	Best Template	Query Coverage	E-Value
CIP-29	2QES	84%	1e-113
Crip-31	2QES	32%	2e-18

2QES

It is Crystal structure of the ribosome inactivating protein PDL4 from *Phytolacca dioica* leaves in complex with adenine.

Molecule	Ribosome-inactivating protein PD-L4
Type	protein
Length	261
Chains	A EC#: 3.2.2.22
Organism	*Phytolacca dioica*
UniProtKB	P84854

The structure of the protein was determined experimentally and the PDB ID is 2QES (Ruggiero *et al*, 2008).

Homology Modeling Results

To this day, homology modeling methods represent one of the most reliable approach to generate a structural model for a protein sequence when at least one suitable template is available (Venclovas *et al*, 2003).

From the fifth Critical Assessment of techniques for protein Structure Prediction (CASP5) experiments, it appears that the critical steps to

obtain a good model are: 1) the selection of the template, 2) the alignment between the target and templates sequences, 3) the modeling of regions not present or structurally different from those in the template and 4) the modeling of side chains (Tramontano and Morea, 2003).

Homology modeling was done using MODELLER9.10 (Agnieszka *et al*, 2005). The modeled structures of the proteins CIP-29 and Crip-31 were viewed under RasMol Version 2.6 (Figure 1 and 2).

Structure Validation Using Saves

Model quality was assessed using various techniques, like geometric or energetic criteria derived from known protein structures. Among the formers, PROCHECK was used to scan a model for unlikely bonds, angles and dihedrals values and for the solvent accessible surface of amino acids (Laskowski *et al*, 1993).

This gave the Ramachandran plot with amino acids in allowed and disallowed regions. These criteria were useful to describe the quality of a protein structure locally, *i.e*. at the residue level to recognize a misfolded model (Eramian *et al*, 2006). Verify3D which is based on a mean force potential (MFP) describing the free energy of interaction between atoms or residue, was used to study statistical preference of amino acids in their environment (Luthy *et al*, 1992).

The structure validation was done using the SAVES web server. The Ramachandran plot was obtained from the SAVES server and validated (Table 1). The modeled structure passed the z-score of WHATIF interface. The structures of the mutated proteins were also validated using SAVES server.

The values of Ramachandran plot is presented in Table 2. The structures that were obtained were used as the receptors against the six ligands of different viral protein. The figure 3 and 4 depict structure validation results of the modeled proteins CIP-29 and Crip-31 using SAVES.

Protein-Protein Interaction Studies Using Cluspro

In silico mutagenesis was carried out in SwissPDB viewer. Figure 5, 6, 7, 8, and 9 are the mutated structures that were modeled by Modeller9.10 of CIP-29 and Crip-31 respectively.

The interaction studies were done by uploading the structures of the two protein in ClusPro 2.0 server. Binding energy of the plant proteins CIP-29 and Crip-31 and the viral proteins obtained from ClusPro 2.0 server are given in Table 3 and Table 4 respectively.

The binding energy values predicted serves as an account of stability and affinity of the viral proteins to bind to the modeled plant proteins. Thus, as the binding energy decreases the mode of interaction and binding capacity increases between the plant proteins and the viral proteins. Table 3 and 4 illustrates the binding energy of the mutated and native proteins to the viral proteins.

Table 1: Structure validation using Ramachandran Plot

Proteins	Ramachandran Plot	Residues In Dis Allowed Region	Z-Score of What If
CIP-29	89.4%	gly12, gly280, ser4, thr29, thr26, asn243, met7, leu284, gln283	-0.556
Crip-31	85.1%	ser284, ser41, arg103, gly58, gly36, val35, leu42, thr130, cys114	-0.075

Table 2: Structure validation of *in silico* mutated proteins using Ramachandran Plot

Proteins	Ramachandran Plot	Residues in Dis Allowed Region
CIP-29 (TYROSINE 271): • ISOLEUCINE • PHENYLALANINE • TRYPTOPHAN	87.9% 87.9% 88.3%	asp83, gly280, thr159, gly100. asp83, gly280, thr159, gly100. asp83, gly280, thr159, gly100.
Crip-31 (PROLINE 188): • CYSTEINE • PHENYLALANINE	85.1% 85.1%	ser284, ser4, arg103, gly36, gly58, cys114, val35, leu42, thr130. ser284, ser41, arg103, gly36, gly58, cys114, val35, leu42, thr130.

Table 3: Binding energy of the plant protein (CIP-29) and the viral proteins obtained from ClusPro 2.0 server

Viral Proteins	Binding –energy values for native CIP-29 to the viral proteins	Tyrosine 271 to isoleucine	Tyrosine 271 to phenylalanine	Tyrosine 271 to tryptophan
Cowpea chlorotic mottle virus (CCMV)	-1508.0	-1261.2	-1259.2	-1260.5
Cowpea mosaic virus (CoMV)	-1070.2	909.9	-909.8	-912.2
Cucumber mosaic virus (CuMV)	-1099.8	-809.9	-827.5	-855.2
Potato yellow mosaic virus (PYMV)	-947.2	-840.6	-926.2	-949.4
Satellite tobacco mosaic virus (STMV)	-1484.7	-1297.6	-1275.5	-1168.3
Tobacco mosaic virus (TMV)	-992.1	-884.9	-884.6	-775.2

Table 4: Binding energy of the plant protein (Crip-31) and the viral proteins obtained from ClusPro 2.0 server

Viral Proteins	**Binding -energy values for native crip-31 to the viral Proteins**	**Proline 188 to phenylalanine**	**Proline 188 to cysteine**
Cowpea chlorotic mottle virus (CCMV)	-1553.5	-1524.5	-1524.5
Cowpea mosaic virus (CoMV)	-1239.6	-1267.2	-1267.3
Cucumber mosaic virus (CuMV)	-1022.6	-1005.1	-1005.0
Potato yellow mosaic virus (PYMV)	-1147.2	-1165.6	-1165.6
Satellite tobacco mosaic virus (STMV)	-1659.2	-1716.7	-1716.8
Tobacco mosaic virus(TMV)	-1065.6	-1108.4	-1108.4

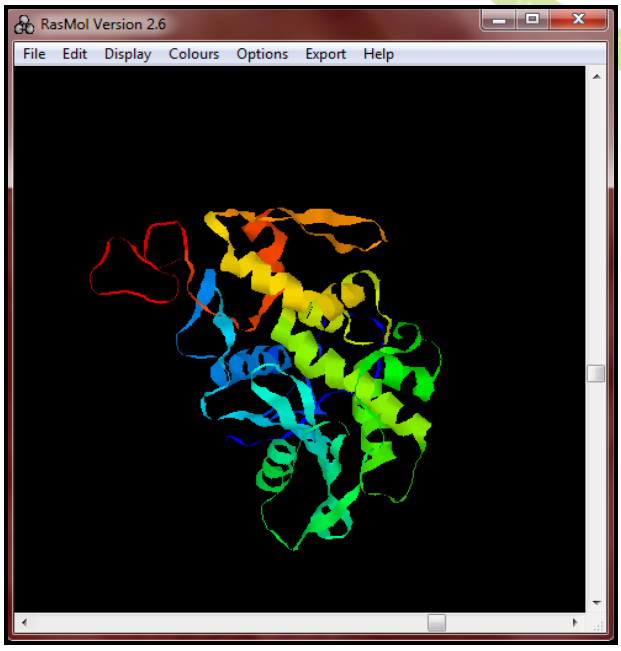

Figure 1: Homology modelling of CIP-29 using Modeller 9.10

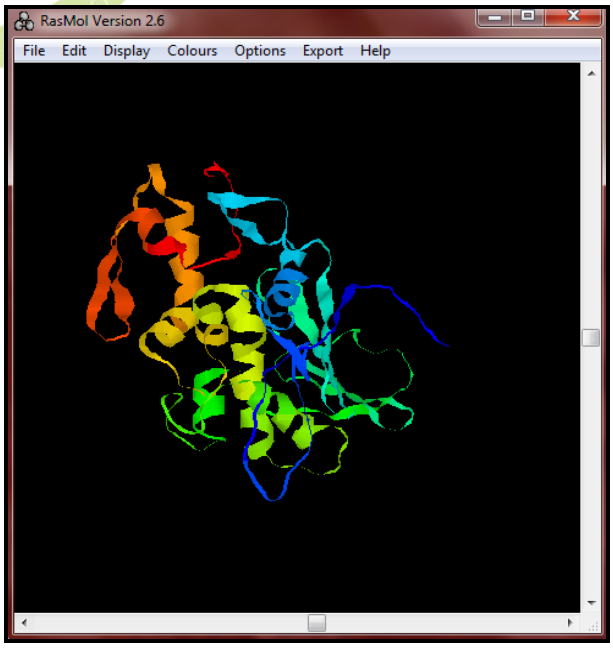

Figure 2: Homology modelling of Crip-31 using Modeller 9.10

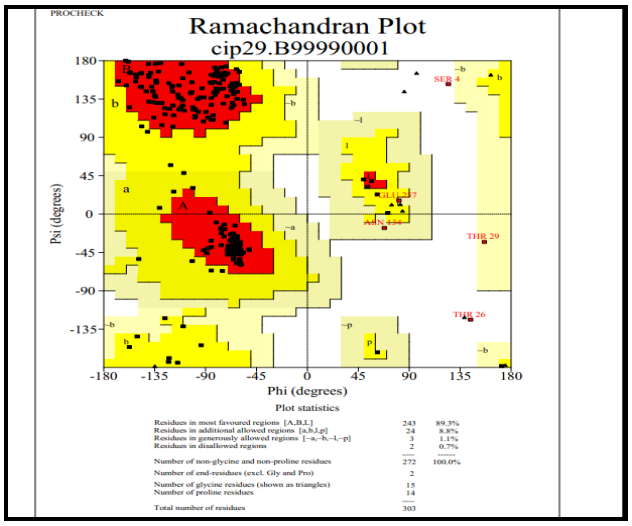

Figure 3: Ramachandran plot of CIP-29 using SAVES server

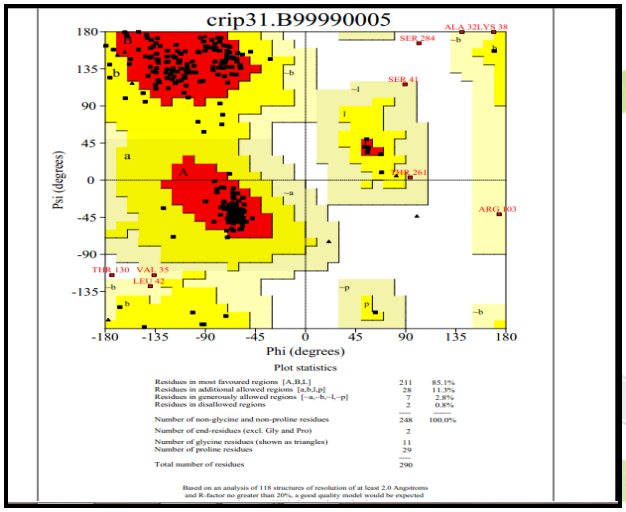

Figure 4: Ramachandran plot of Crip-31 using SAVES server

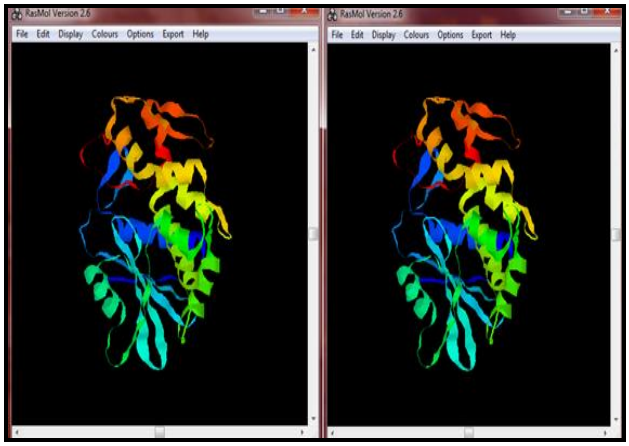

Figure 5 & 6: Homology modelling of mutated CIP-29 (TYROSINE 271) into isoleucine and phenylalanine using Modeller 9.10

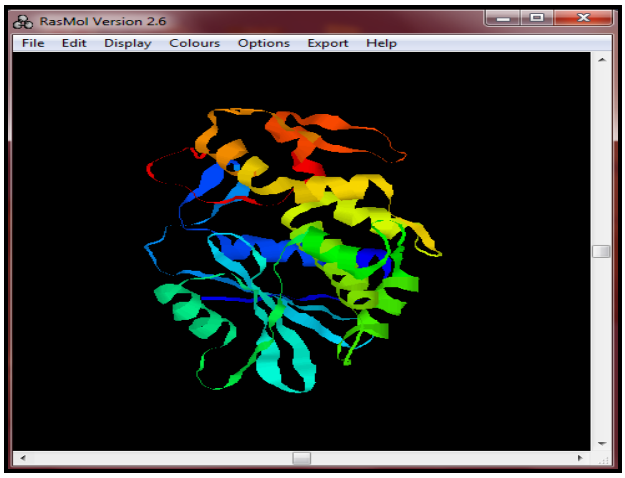

Figure 7: Homology modelling of mutated CIP-29 (TYROSINE 271) into tryptophan using Modeller 9.10

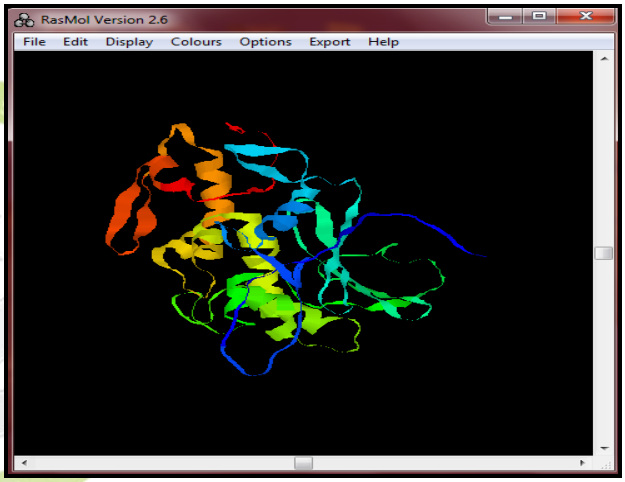

Figure 8: Homology modelling of mutated Crip-31 (PROLINE 188) into cysteine using Modeller 9.10

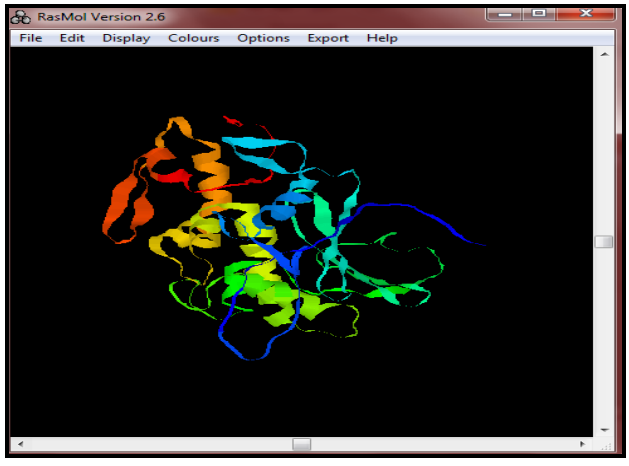

Figure 9: Homology modelling of mutated Crip-31 (PROLINE 188) into phenylalanine using Modeller 9.10

DISCUSSION

Mutation in CIP-29 shows it interacts with PYMV with very minimum binding energy out of six viral proteins. This shows that the protein is effective in its native state than in the mutated state. Mutation in Crip-31 showed minimum binding energy against four viral proteins (CoMV, PYMV, STMV, and TMV) among the economically available viral proteins which depicts that the protein Crip-31 interacts in broad spectra with several viral proteins.

CONCLUSION

In this study the antiviral property of the proteins CIP-29 and Crip-31 of *Clerodendrum inerme* against economically important viruses was carried out by *in silico* analysis. Tools like Modeller9.10, SAVES, ClusPro2.0, protein interactions calculator (PIC), SwissPDBviewer, was used to carry out the study. Protein interaction studies against different viral coat and the replicase protein was done. *In silico* analysis of CIP-29 proteins against five viral coat proteins and one replicase protein revealed that the proteins in the native state binds effectively than the protein in the mutated state. In case of Crip-31, mutated form binds effectively with four viral proteins (CoMV, PYMV, STMV, and TMV) and the native form is more efficient against CCMV and CuMV.

REFERENCES

1. Gurudeeban, S., Satyavani, K., Ramanathan, T., Umamaheshwari, G., & Shanmugapriya, R. (2010). Antioxidant and radical scavenging effect of Clerodendrum inerme (L). *World Journal of fish and marine sciences*, *2*, 66-69.
2. Anitha, R., & Kannan, P. (2006). Antifungal Activity of Clerodendrum inerme (L). and Clerodendrum phlomidis (L). *Turkish Journal of Biology*, *30*(3), 139-142.
3. Olivieri, F., Prasad, V., Valbonesi, P., Srivastava, S., Ghosal-Chowdhury, P., Barbieri, L., & Stirpe, F. (1996). A systemic antiviral resistance-inducing protein isolated from Clerodendrum inerme Gaertn. is a polynucleotide: adenosine glycosidase (ribosome-inactivating protein). *FEBS letters*, *396*(2), 132-134.
4. Prasad, V., Srivastava, S., & Verma, H. N. (1995). Two basic proteins isolated from Clerodendrum inerme Gaertn. are inducers of systemic antiviral resistance in susceptible plants. *Plant science*, *110*(1), 73-82.
5. Pandey, R., Verma, R. K., & Gupta, M. M. (2005). Neo-clerodane diterpenoids from Clerodendruminerme. *Phytochemistry*, *66(6)* 643-648.
6. Kozakov, D., Hall, D. R., Beglov, D., Brenke, R., Comeau, S. R., Shen, Y., & Vajda, S. (2010). Achieving reliability and high accuracy in automated protein docking: ClusPro, PIPER, SDU, and stability analysis in CAPRI rounds 13–19.*Proteins: Structure, Function, and Bioinformatics*, *78*(15), 3124-3130.
7. Venclovas, C., Zemla, A., Fidelis, K., & Moult, J. (2003). Assessment of progress over the CASP experiments. *Proteins: Structure, Function, and Bioinformatics*, *53*(S6), 585-595.
8. Tramontano, A., & Morea, V. (2003). Assessment of homology-based predictions in CASP5. *Proteins: Structure, Function, and Bioinformatics*, *53*(S6), 352-368.
9. Read, R. J., Brayer, G. D., Jurasek, L., & James, M. N. (1984). Critical evaluation of comparative model building of Streptomyces riseus trypsin. *Biochemistry*, *23*(26), 6570-6575.
10. Laskowski, R. A., MacArthur, M. W., Moss, D. S., & Thornton, J. M. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography*, *26*(2), 283-291.
11. Eramian, D., Shen, M. Y., Devos, D., Melo, F., Sali, A., & Marti-Renom, M. A. (2006). A composite score for predicting errors in protein structure models. *Protein Science*, *15*(7), 1653-1666.

12. Liithy, R., Bowie, J. U., & Eisenberg, D. (1992). Assessment of protein models with three-dimensional profiles. *Nature, 356*(6364), 83-85.
13. Chmiel, A. A., Bujnicki, J. M., & Skowronek, K. J. (2005). A homology model of restriction endonuclease SfiI in complex with DNA. *BMC Structural Biology*, *5*(1), 2.
14. Ahmad, P., & Prasad, M. N. V. (2011). *Environmental adaptations and stress tolerance of plants in the era of climate change*. Springer. *Springer Science+Business Media*, LLC 2012.
15. Bohnert, H. J., & Cushman, J. C. (2000). The ice plant cometh: lessons in abiotic stress tolerance. *Journal of Plant Growth Regulation*, *19*(3), 334-346.
16. P. Narayanasamy. *Immunology in plant health and its impact on food safety*. 1. Food Products Press.1- 412.

