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Research Article

Homology modeling, structure and active site prediction of stem rust resistant gene Sr22 in wheat cultivars

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Abstract

Protein structure and function is very important to study before gene transformation. Protein homology modeling is very important tool used to predict the structure of the protein. *Sr22* gene codes for a Sr22 protein which confers resistance against *Puccinia graminis*. The homology modeling of Sr22 protein was obtained by using SWISS-MODEL web tool. Then the predicted model of Sr22 protein was superimposed to check the validation through errat. Errat score for this model was 77% which was further improved up to 80% by doing loop optimization of Sr22 using similar tool. 3D model of stem rust resistant protein was further used to analyze the activity of the protein. Active sites of Sr22 protein were detected by using ADDS (Automated active sites detection, docking and scoring) software. Total of 17 active sites were identified and active sites with the highest score were used in further studies. The information achieved may be useful to understand the mechanism resistance to stem rust disease in wheat which may further help to manipulate for sustainable resistance.

Keywords: Active site prediction; Homology Modeling; *Puccinia graminis*; Sr22 protein; Stem Rust

Introduction

Puccinia graminis f. spp. Tritici Pers. causes stem rust disease in wheat crop. Sr22 is a gene that provides resistance against the fungus. This disease has the potential to destroy the whole crop and is an immediate threat to world wheat production. **[1]**. The *Sr22* gene was first discovered in diploid wheat species *Triticum monococcum* **[2]** but during the course of evolution from diploid to hexaploid, this gene was lost. It was incorporated into tetraploid and hexaploid wheat through interspecific hybridizations and in commercial varieties by breeding. *Sr22* is resistant against *Puccinia graminis* but it also lowers the yield and delays in

heading date due to some other genes associated with the *T. monococcum* ssp. *boeoticum* chromosome segment carrying *Sr22*. This drawback has limited its use in breeding [3, 4].

Sr22 is reported in diploid wheat cultivars. It is a large gene having a size of 2823 bp of the coding region. Before the introduction of *Sr22* gene in commercial wheat from the wild genome of diploid wheat, it is necessary to understand the product of the gene and the pathways in which it is involved. Bioinformatics tools are the best sources to understand the function and behavior of the Sr22 protein. This article aimed to model the Sr22 protein and predict its active binding sites *in silico*. The information achieved will be used to identify the appropriate effectors for Sr22 protein first *in silico* and then *in vitro* [5].

Material and methods

Sequence retrieval

The sequence of Sr22 protein was retrieved from the NCBI sequence database (Accession # LN883752.1).

Protein homology modeling

Homology modeling of Sr22 proteins was done by using SWISS-MODEL web bioinformatics tool (https://swissmodel.expasy.org/). It is a web tool used for the homology modeling of protein three-dimensional structure [6].

Model evaluation

The 3D model of Sr22 protein was evaluated by ERRAT. This model helps to analyze the statistics of non-bonded interactions among types of atom. This model also facilitates to plot the value of the error function versus position of a 9-residue sliding window. All these results were obtained by comparing values with statistics originated from highly refined structures [7].

Active site prediction

ADDS (Automated active sites detection, docking and scoring) was used to predict active sites [8] of Sr22 protein. The accuracy of this software was checked by the molecules of known active sites.

Result and discussion

Protein model

The homology modeling of Sr22 protein of wheat (*Triticum aestivum*), which is encoded by stem rust resistant gene *Sr22* was done by SWISS-MODEL [9]. The model was constructed using the amino acid sequence as a template for Sr22 protein. Five models were generated and the model with maximum GMQE (Global Model Quality Estimation) was selected as the best model (Figure 1 & Table 1).

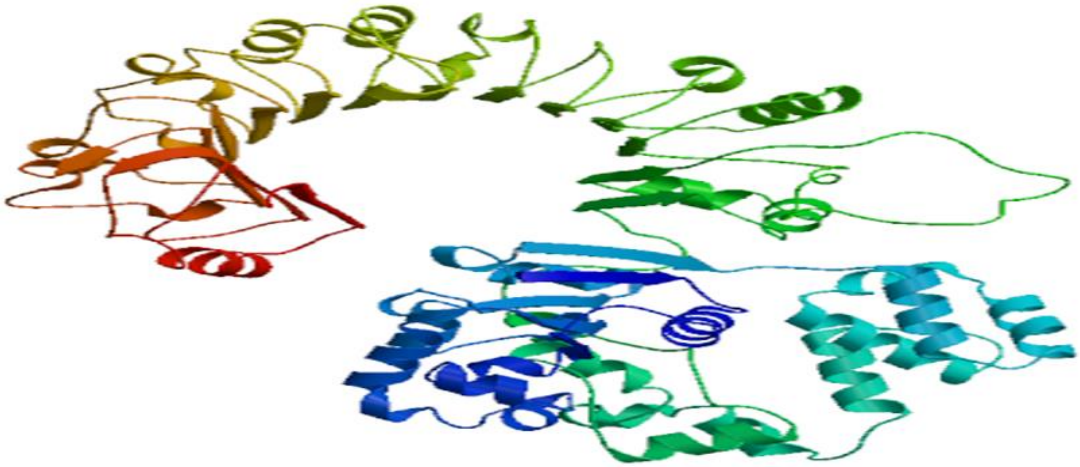

Figure 1. Three- dimensional protein structure of Sr22 best predicted by SWISS-MODEL

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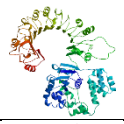
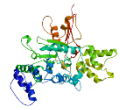
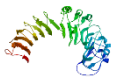
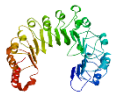
Table 1. Five best models of Sr22 protein predicted by SWISS-MODEL with their quality parameters

Model # 01	Model	Oligo-State	Ligands	GMQE	QMEAN
1		monomer	None	0.34	-6.43
2		monomer (matching prediction)	None	0.20	-7.17
3		monomer (matching prediction)	None	0.20	-8.21
4		monomer	None	0.16	-7.27
5		monomer	None	0.15	-6.80

Model Evaluation

The selected best model was evaluated by ERRAT. The best model score of Sr22 protein was 77%. The score was further improved up to 80% by doing loop optimization of Sr22 protein using a similar tool (Figure 2).

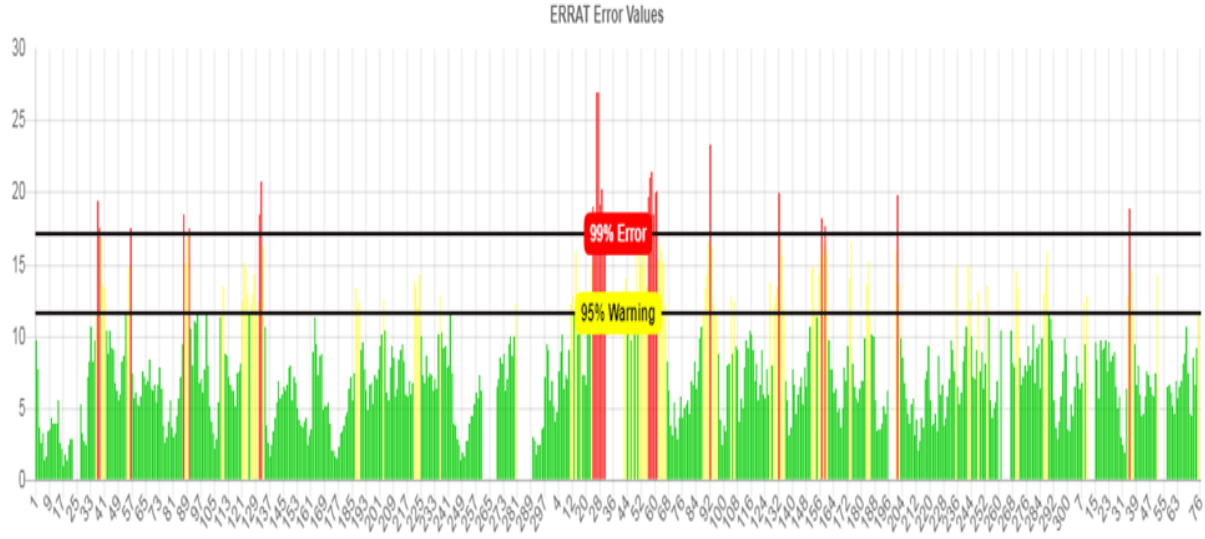


Figure 2. Validation of a predicted model of Sr22 protein by ERRAT software

Active site prediction

Active sites of wheat Sr22 protein was predicted using ADDS (Automated active sites detection, docking and scoring) [10]. Total of 37 cavities were predicted initially but the only one pocket with the highest score was selected for further analysis (Figure 3).

The Sr22 protein haplotype of PI 306540 carries the same amino acids as the resistant alleles at three of the four positions that discriminate the resistant and susceptible Sr22 protein haplotypes. There are 8 polypeptide binding interaction sites in the protein (75, 79, 82, 111, 114, 117-119, 121-123 and 125-126) (Table 2).

Sr22 gene is a high potential rust resistant gene but unfortunately very less studied. Its functions are still unknown [12]. Only one domain is matched with an existing protein database. On the other hand, this protein has 7 Leucine-rich regions and 8 binding sites which make it highly effective against the *Puccinia graminis*. Leucine-rich regions are known to identifying the fungus proteins and activate the defense mechanism [13].

The binding sites have the ability to bind with GTPase Activating Protein 2 (RanGAP2). Initially, RanGAP2 was identified as a regulator of cell division and nucleocytoplasmic trafficking. Later it was discovered that it also plays an important role in interacting with the Coiled-Coil domain [14]. Coiled-Coil domain is a structural motif in protein in which 2 to 7 alpha helix structures of protein coiled together to make a rope-like structure [15]. *Sr33* another gene of this family that confirms the resistance against wheat stem rust also has such Coiled-Coil domain. This Coiled-Coil domain presence in *Sr33* predicts that *Sr22* will also have to confirm resistance against *Puccinia graminis* [16].

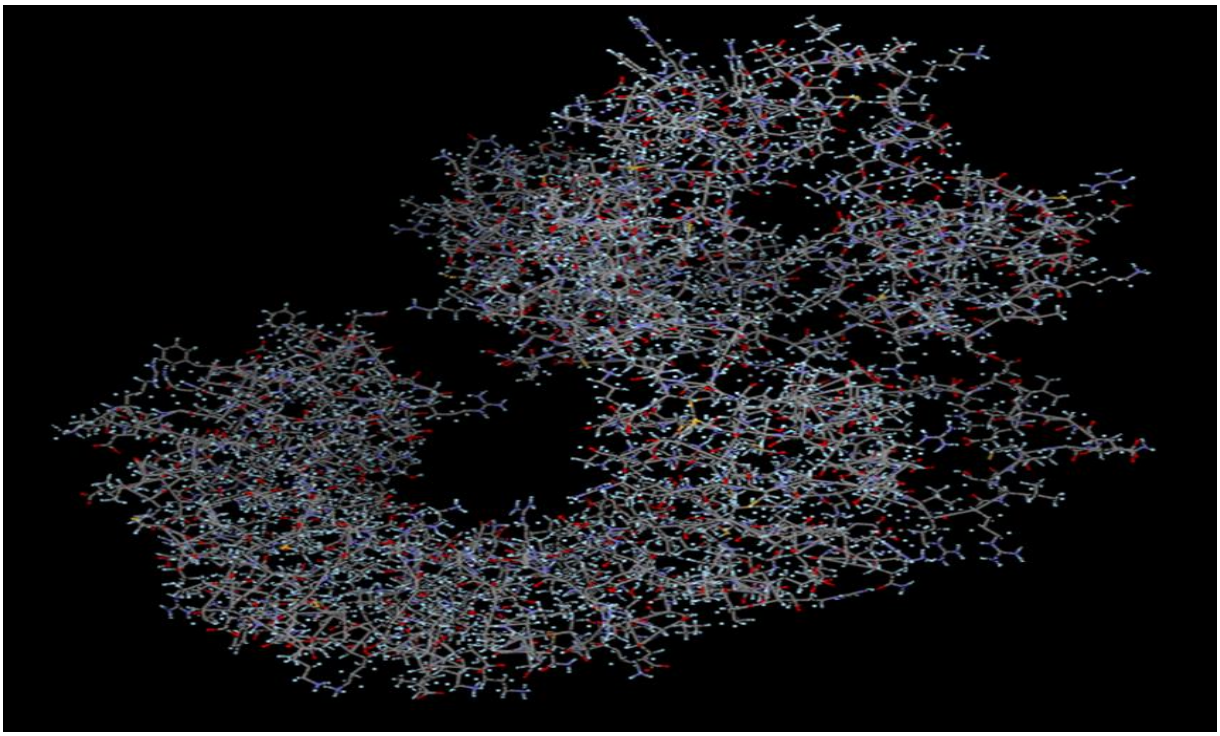


Figure 3. The three-dimensional structure of active sites of Sr22 protein generated by AADS tool. The different colors show different atoms as a white color for carbon, blue for nitrogen, red for oxygen purple for hydrogen

Table 2. Sr22 protein with function associated sites [11]

Sr. No	Regions	Location	Function
1	Coiled-coil domain	11-139	Resistant domain
2	polypeptide binding	75	RanGAP2 interaction site
3	polypeptide binding	79	RanGAP2 interaction site
4	polypeptide binding	82	RanGAP2 interaction site
5	polypeptide binding	111	RanGAP2 interaction site
6	polypeptide binding	114	RanGAP2 interaction site
7	polypeptide binding	117-119	RanGAP2 interaction site
8	polypeptide binding	121-123	RanGAP2 interaction site
9	polypeptide binding	125-126	RanGAP2 interaction site
10	NB-ARC domain	176-458	
11	Leucine-rich repeat (LRR)	<524 - >727	Provide recognition of pathogen products of avirulence (AVR) genes
12	Leucine-rich repeat (LRR)	567-597	Provide recognition of pathogen products of avirulence (AVR) genes
13	Leucine-rich repeat (LRR)	598-620	Provide recognition of pathogen products of avirulence (AVR) genes
14	Leucine-rich repeat (LRR)	621-641	Provide recognition of pathogen products of avirulence (AVR) genes
15	Leucine-rich repeat (LRR)	642-662	Provide recognition of pathogen products of avirulence (AVR) genes
16	Leucine-rich repeat (LRR)	725-769	Provide recognition of pathogen products of avirulence (AVR) genes
17	Leucine-rich repeat (LRR)	770-793	Provide recognition of pathogen products of avirulence (AVR) genes

Conclusion

The advantage of this study is that the gene *Sr22* is itself present in the wheat genome in old and wild cultivars. So there is a possibility of no issues with the metabolites of the transgenic plant as compared to non-transgenic except the protein of *Sr22*.

Authors' contributions

Conceived and designed the experiments: S Bashir & SA Bukhari, Performed the experiments: S Bashir, Analyzed the data: S Bashir, SA Bukhari & MU Rahman, Contributed materials/ analysis/ tools: SA Bukhari, MU Rahman & M Ali, Wrote the paper: S Bashir.

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