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# In Silico Analysis and Function Prediction of *Sr22* Gene Product as Stem Rust Resistant Protein

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## Abstract

Wheat is predominantly used as a staple food all over the world. Stem rust resistance by *Sr22* gene is researched and confirmed before but in the current study, its molecular function is predicted by using different in-silico tools such as ExPASy-Translate, ExPASy- PortParam, and InterProScan. We investigated the in-silico functional compositions of *Sr22* protein, and the results revealed that it belongs to the Plant Disease Resistant family and is comprised of Leucine-rich repeats at C-terminal and Adenosine 5'-Diphosphate Binding domain at N terminal. As an ADP binding protein, it can arrest fungal growth by inhibiting the function of nucleic acid. Moreover, *Sr22* protein is also known to involve in regulating programmed cell death (PCD).

**Keywords:** Sr22, Stem Rust, ExPASy-Translate, ExPASy- PortParam, and InterProScan

## Introduction

Wheat crop is one of the major food sources that is grown worldwide. Stem rust is a common plant disease, caused by the fungus (*Puccinia graminis*), for wheat cultivation that results in its reduced annual production. The wheat *Sr22* gene holds the potential to provide tolerance against stem rust This disease has devastating impacts on the whole crop growth and wheat production which is an immediate concern to the world. The *Sr22* gene was first discovered in the diploid wheat species *Triticum monococcum*, but during its evolution from diploid to hexaploid species, this gene is lost (Steuernagel et al, 2016; Hatta et al, 2021). It was further incorporated into tetraploid and hexaploid wheat species by utilizing conventional breeding methods to produce commercial varieties that are mostly preferred by the breeders.

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This conventional breeding tool results in crop resistance against *Puccinia graminis*, nevertheless it also revealed to lower the wheat yield and delays in the reproductive phase due to some genes linkage within the same chromosome (Bashir et al, 2019). Although the ultimate role of the *Sr22* gene is to endorse resistance against the stem rust disease, its molecular basis is still unclear. The *Sr22* protein function prediction based on the molecular basis is desirable to make a perspective about its transformation in commercial cultivars (Bukhari et al, 2018). This study focused to determine *Sr22* protein function by using different *in-silico* tools. The concluded results will be beneficial for wheat researchers and breeders to use appropriate strategies of genetic engineering to enhance the wheat crop resistance against stem rust.

## Methods

This study encompasses the in-silico function prediction of the *Sr22* gene without any wet lab experiments' by the author(s). The gene sequence of the *Sr22* (2823 bp) was retrieved from NCBI (accession no: MH512000) reported by Bukhari et al, (2020). While it's respective protein sequence (940 Amino acids) was obtained by using ExPASy-Translate tool (Gasteiger et al, 2003), similarly, protein physicochemical properties were predicted by using ExPASy-PortParam server (Sahay et al, 2020) which includes following parameters like molecular weight, chemical formula, atomic composition in the protein, theoretical *pI*, half-life, instability index, grand average of hydropathicity (GRAVY), and aliphatic index (Gasteiger et al, 2003). InterProScan is a resource database that permits scan both nucleotide and protein sequences across different InterPro databases. Moreover, it also provides protein functional analysis, classification into different families, presence of domains, and active sites residues within the protein orthologue (Quevillon 2005; Blum et al, 2021).

## Results and Discussions

Its predicted molecular weight and formula (by ExPASy- PortParam) was 106173.92 g and 106.19 kDa respectively, while its chemical formula was $C_{4703}H_{7645}N_{1279}O_{1412}S_{46}$. As isoelectric point (IP) is a unique property to predict solubility and mobility in an electro-focusing system. The computed IP value of *Sr22* protein was 5.83 which is less than 7, this indicates the protein has a stable and compact structural configuration. The measured instability index value of *Sr22* protein was 44.17 which lies in the range of reported value (18.43 to 45.31), which concludes that this is a stable protein. ExPASy- PortParam predicted *Sr22* protein as a thermostable protein due to the increased number of aliphatic side chains and high aliphatic index (AI) value (103.11) thus it contains 37.44% aliphatic amino acids. Moreover, the grand average hydropathy (GRAVY) value for *Sr22* protein or peptide is calculated as the sum of hydropathy values of all the amino acids included, divided by the number of residues present in the sequence. The (GRAVY) was found to be – 207, this lower value shows the possibility of better interaction with water.

InterProScan prediction shows that Sr22 protein belongs to a Disease Resistant Protein family (DRP), this information matches with the existing protein's secondary structure of Disease resistance proteins members. The disease-resistant proteins are mostly prevalent in cereal plants. They possess C- terminal Leucine-rich repeats which authenticate its disease resistance property, it also contains a nucleotide-binding site at the N-terminal position. The DRP family members possess 1895 resistant genes that are distributed among 12 plant species. These species include *Zea mays*, *Vitis vinifera*, *Trypanosoma brucei*, *Solanum lycopersicum*, *Populus trichocarpa*, *Physcomitrella patens*, *Oryza sativa*, *Leishmania major*, *Glycine max*,

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*Entamoeba histolytica*, *Brachypodium distachyon*, and *Arabidopsis thaliana* respectively (Song et al, 2021).


**Figure 1:** Results of different domains by different databases

InterProScan server predicts three domains that may be involved to control the specific function of *Sr22* protein. The first domain is NB-ARC (Nucleotide Binding adaptor) domain which is mostly present in bacteria and also in cereal plants. This domain offers a unique ability in binding and hydrolyzation of ATP into ADP. The purification of this domain protein along with ADP revealed to act as a switch between ATP and ADP and facilitate the apoptosis of pathogens (Steele et al, 2019).

The second domain is a virus X resistance protein (RX) which is located on the N terminal site of the protein. The third domain is the coiled-coil (CC) domain which aid in the recognition of specific pathogens. While the CC domain is activated by Ran GTPase Activating Protein 2 (RanGTP2) that is further triggered by fungus protein RanGTP1 and is produced during cell division (Carpentier et al, 2013).

InterProScan predicts two Homologues super-families in *Sr22* protein. The leucine-rich repeat (LRR) domain superfamily includes tyrosine kinase receptors. These receptors are regulatory in a different field but specifically, it is responsible for cell to cell communication and finally apoptosis. The second super-family contains P-loop ATPase domains, which are also used for the apoptosis of the cell (Leipe et al, 2003).

The IntroProScan is also used to predict the molecular function of Adenosine 5'-Diphosphate Binding (ADP) protein. This binding protein inhibits the production of nucleic acid which in return affects pathogen growth. The molecular function is described in the following flow chart (Figure 1).

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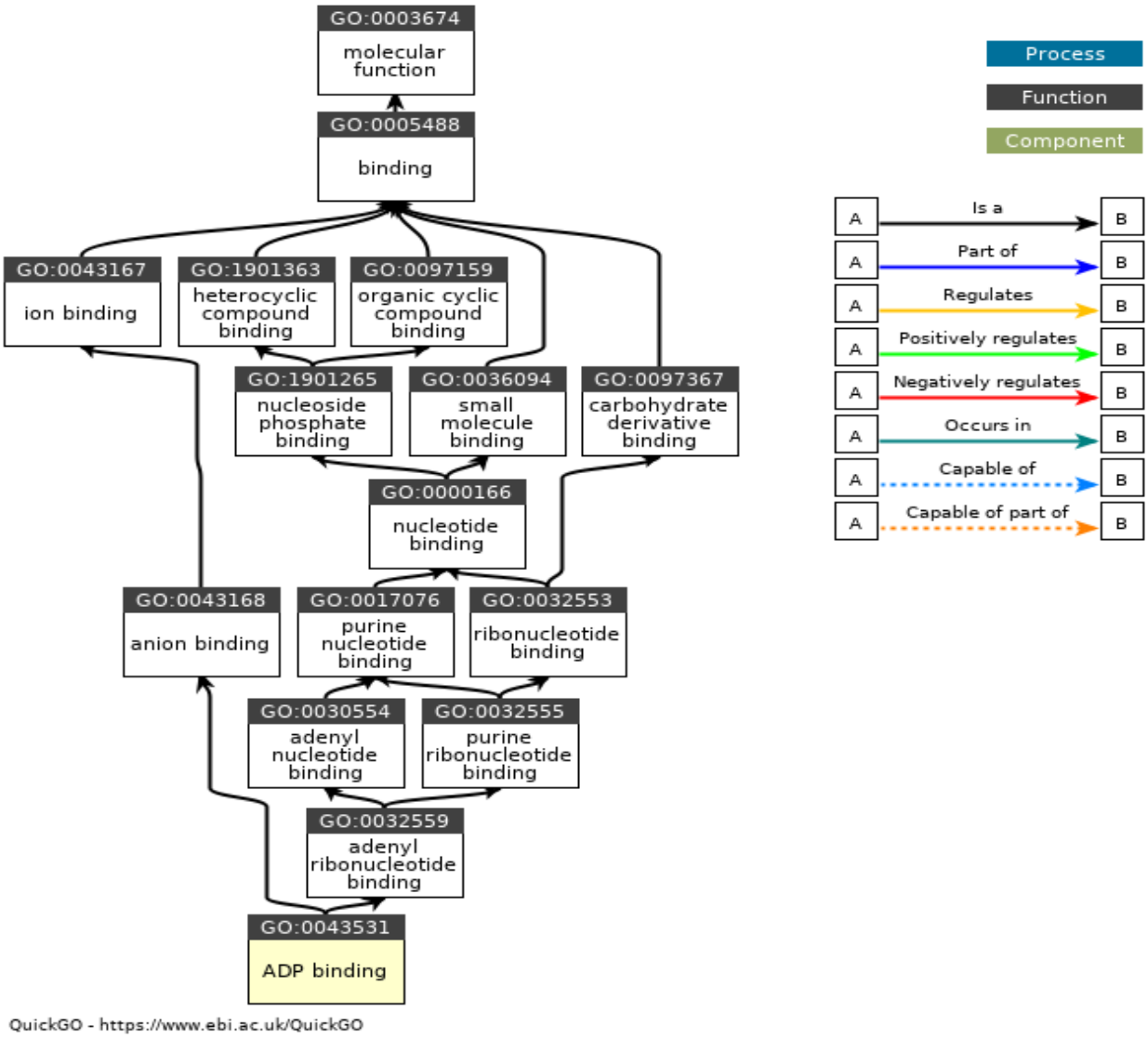


**Figure 2:** Function prediction of Sr22 protein by IntroProScan

## Conclusion

Sr22 is a resistant gene code for a protein that possibly help to reduced growth of invading fungal pathogen by DNA binding proteins. It is also involve in the programmed cell death of the fungal cell and the infected cell of the host as well.

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## Declaration

The authors declare that they have no conflict of interest.

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