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Investigating the interaction between some of Bipolaris sorokiniana's toxins and the Gα Subunit of the Wheat G-Protein using bioinformatics tools

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Abstract

Spot blotch disease of wheat, caused by the fungus *Bipolaris sorokiniana* (Sacc.) Shoem., produces several toxins like: prehelminthosporol, helminthosporol, helminthosporic acid, sorokinianin, Bipolaroxin. These toxins interact with the plant and thereby increase the symptoms of small dark lesions and huge yield losses in different regions around the world so there is an urgent need to decipher the molecular interaction between wheat and those toxins for in-depth understanding of host–pathogen interactions. In the present study, we have modeled the three-dimensional structure of G-protein alpha subunit from *Triticum aestivum* as G-protein was shown that it is an important player behind the resistance to many plant diseases. Molecular docking studies were performed using the active site of the modeled G-protein alpha subunit from *T. aestivum* and some of fungus's toxins followed by molecular dynamics (MD) simulation studies to explore the stability, conformational flexibility, and dynamic behavior. Protein-ligand interaction study revealed one H-bond formed by Lys302 and hydrophobic contacts formed by Tyr159, Gly162, Val167, Asp256, Gln257 and Ile298 with prehelminthosporol, Protein-ligand interaction study revealed H-bond formed three H-bonds formed by Tyr159, Gly162, and Asp256 and hydrophobic interactions formed by Ser160, Cys161, Ser162, Ile298, Lys302 and Val306 with helminthosporic acid. Protein-ligand interaction study revealed two H-bond formed by His172, Arg301 and hydrophobic interactions by Tyr159, Pro168, Asp169, Ile298 and Lys302

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with sorokinianin. Protein-ligand interaction study revealed H-bond formed six H-bonds mainly formed by Glu29, Ser30, Lys32, and Ala177. In addition to H-bonds, hydrophobic contacts formed by Gly28, Gly31, Ser33, Thr34, Arg78, Val179, Thr181 and Gly209 with Bipolaroxin were also observed.

I. INTRODUCTION

Studying plant pests is an urgent need to ensure food security in the face of the continuous increase in global population. As wheat is one of the most important crops cultivated and consumed worldwide, managing the pests affecting this crop is a priority for agricultural experts. However, with the advancements of computational methods nowadays, the use of bioinformatics tools has become necessary to study various pests affecting plants in general and wheat in particular. This is in order to obtain a broader and more comprehensive understanding of the interactions between the pathogen and the host, which contributes to better management of different pests. Therefore, this study aims to utilize Molecular Docking and Molecular Dynamics (MD) simulations to investigate the interaction between certain toxins produced by the fungus *Bipolaris sorokiniana* and the alpha subunit of the G-protein, which will be modeled. This is for gaining a deeper understanding of the mechanism of this interaction at the molecular level.

crop

Wheat (*Triticum aestivum*) is considered one of the most widely cultivated crops in the world. Its production exceeded 760 million tons in 2020, covering an area of 219 million hectares of land globally [15].

pathogen

Bipolaris sorokiniana (Sacc.) Shoemaker (*syn. Helminthosporium sativum teleomorph: Cochliobolus sativus*), a hemibiotrophic phytopathogenic fungus, is well-known for affecting cereal production, including wheat, barley, rice, and oats [1]. This phytopathogen can infect several plant organs (e.g., roots, stems, leaves, seeds) and causes diseases such as root rot, spot blotch, head blight, seedling blight, and black point [2]. Spot Blotch (SB) is the most devasting disease caused by this phytopathogen, which is characterized by the appearance of small dark brown leaf lesions caused by the release of toxins and cell wall degrading enzymes during the necrotrophic stage of the infection [27]. SB is one of the most important foliar pathogens of wheat causing 2.7 to 100% losses in various countries depending on varieties [23]. Globally, an estimated 25 million ha of wheat land is affected by SB [55].

Bipolaris sorokiniana produces a range of toxic compounds commonly known as phytotoxins, which include prehelminthosporol, helminthosporol, helminthosporic acid, and sorokinianin [23]. These compounds are known to inhibit seed germination and hypocotyl/radical growth [38]. Jahani et al. identified a new compound, ‘Bipolaroxin’ which produces yellow and/or necrotic lesions when placed on the wheat leaves [23]. It was shown that helminthosporol displayed a strong phytotoxic effect on lettuce seed germination [38]

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and it is considered to be responsible for inducing spot blotch symptoms, it seems to play an important role in pathogenesis by killing or weakening plant cells prior to invasion by the growing hyphae [12]. A study indicated that helminthosporol drastically affects the membrane permeability of these organelles to protons and substrate anions, inhibiting the mitochondrial oxidative phosphorylation, the photophosphorylation in chloroplasts, and the proton pumping across the cell plasma membrane [5]. helminthosporic acid displayed weak necrotic activity against wheat leaves [34]. More recently, the prehelminthosporol (PHL), has been thought to be principally responsible for phytotoxicity affecting the 1,3-β-glucan synthase activity on the plasma membrane and disrupting cell membranes [12].

Symptoms of SB

The dark brown necrotic spots (boat shaped) occur on the coleoptiles, leaves, crowns, stems, and roots with or without yellow halo around these. Darkening of the sub crown internode is a characteristic symptom. Lesions on the leaves start as a few mm that extend as elongated dark brown spots greater than 1-2 cm [7]. A yellowing due to toxin production is sometimes observed extending from the lesion. Later such spots coalesce each other thus result blight on large leaf portion. As the disease progresses the spots join together forming large blotches that cover the leaves and eventually killing it. On leaves, conidia develop readily under humid conditions and spread over several centimeters before coalescing and inducing the death of the leaf tissue. An abundant production of conidia can be observed on old lesions under humid conditions and chlorotic streak is sometimes seen diffusing from the border of the lesion as a result of toxin production [4-12]. Stunting and reduced tillering may be observed in heavily infected plants which may lead to premature death, resulting in white heads. Kernels become shriveled and roots become dark brown and rotted. Yields may be reduced due to root rot even though symptoms are not well-developed [10].

Epidemiology of SB

Spot blotch is seed transmitted disease and its conidia survive in the soil. Spot blotch is the most widely distributed disease of cereal crops (especially wheat and barley) in the

subtropics, mainly in south Asia and some parts of South America. Ideal conditions for spot blotch development on the leaves are high relative humidity with high temperature and long periods (more than 12 to 18 hours) of leaves wetness caused by rainfall, irrigation, fog or dew. Conidia present on infected stubble

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and on the soil surface are dispersed by wind and initiate lesions on the leaves and stems later in the season. The most important factor, temperature plays a key role coupled with high humidity. Moderate to warm temperature range (18˚C to 32˚C) favours the growth of *B. sorokiniana*. Even at the end of the monsoon and in absence of rainfall, high relative humidity arising from high levels of soil residual moisture along with foggy days allows long hours of wetness on leaf blades that can last until late January in Indo-Gangetic Plains, creating ideal conditions for the establishment and multiplication of pathogen. There are various cycles of conidia production during the cropping season which lead to secondary infections after spreading through wind and water drops. Many scientists reported that disease was more severe at 28°C than at lower temperatures. Epidemiological studies have shown that timely sowing avoids the physiological stress that often coincides with the flowering stage which in turn reduces spot blotch [14].

Yield losses by SB

Spot blotch has been considered as a major constraint to wheat yields in South Asia due to reduction in 1000-grain weight and grain yield. In Turkey, *B. sorokiniana* has been observed to be widespread in the sub crown internodes and crowns of wheat. The pathogen also causes grain yield losses up to 10, 15, and 20 per cent through common root rot and seedling blight in countries like Scotland, Canada, and Brazil, respectively. At higher latitudes, such as the Canadian and US prairies and in parts of Australia B. sorokiniana is a dominant pathogen among fungi, causing common root rot and resulting in losses of up to 19 per cent [16-17].

G-protein

Recently, an important player behind the resistance to necrotrophic fungi was discovered, i.e., heterotrimeric G-protein [26-48]. Heterotrimeric G-proteins are universal signal transducers composed of α, β, and γ subunits coupled with a plethora of receptors commonly known as G-protein-coupled receptors (GPCRs) found in all eukaryotic organisms, including plants, fungi, and animals [32]. Moreover, heterotrimeric G-protein signaling in plants differs from that in animals. Plants have only a small number of genes encoding the G-protein subunits; Only 1 canonical G subunit gene has been identified in monocots and dicots, as compared to 17 in mammalian species [24]. The higher plant Arabidopsis has only one canonical G-protein α-subunit (Gα), one β-subunit (Gβ), and three γ-subunits (Gγ) [45] while only two Gγ were found in monocot rice species [56]. Classically, the G-protein heterotrimer is activated by the cell-surface receptors (GPCRs) that trigger the Gα subunit of the heterotrimer to release GDP, thus enabling the Gα subunit to bind GTP [21]. The plant heterotrimeric G-protein has been implicated in auxin, gibberellin, and abscisic acid signaling, light responses, cell division ion-channel regulation [3-33] and in the host–pathogen interaction [32]. A number of reports suggest the direct roles of G-proteins in plant defense against a variety of pathogens [44-47-49].

Trusov et al. claimed that jasmonic acid (JA) was involved in heterotrimeric G-protein mediated resistance against necrotrophic pathogen in Arabidopsis [47]. Recent studies, mostly in Arabidopsis and rice, have

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revealed very important roles for G proteins in plant resistance to fungal pathogens [49]. All three regular subunits of the Arabidopsis G-protein play roles in disease resistance [21]. Additionally, it is shown that Gα is involved in disease resistance of rice [44]. studies suggest that the role of G-proteins in plant disease resistance depends on the type of pathogen (necrotropic vs. biotrophic and bacterial vs. fungal) and that the different G-protein subunits may have different functions [21].

Host-pathogen interaction

Malviya et al. employed the molecular docking technique coupled with MD simulations to infer the probable mode of binding of Bipolaroxin to the Gα and Gβ subunits and how Bipolaroxin modulates the physio-biochemical and molecular mechanisms in wheat. They noticed that the Bipolaroxin ligand prefers to bind in the cavity formed of helical and Ras domain of G-alpha subunit and Protein–ligand interaction study revealed six H-bonds mainly formed by Glu29, Ser30, Lys32, and Ala177 of G-alpha with Bipolaroxin while in the beta subunit, the residues of the core beta strand domain participate in the ligand interaction where Lys256, Phe306, and Leu352 formed seven H-bonds with the ligand Bipolaroxin. In addition to H-bonds, hydrophobic contacts were also noticed to

be formed of Gly28, Gly31, Ser33, Thr34, Arg178, Val179, Thr181, and Gly209 of G-alpha and Trp74, Ala305, Phe306, Ile308, Leu350, Gly351, and Ser354 of G-beta with Bipolaroxin [11].

Molecular Modelling:

Homology modelling is one of the computational structure prediction methods that are used to determine protein 3D structure from its amino acid sequence. It is considered to be the most accurate of the computational structure prediction methods. It consists of multiple steps that are straightforward and easy to apply. There are many tools and servers that are used for homology modelling. There is no single modelling program or server which is superior in every aspect to others [30].

Molecular Docking:

Molecular docking is to simulate the optimal conformation according to the complementarity and pre-organization, which could predict and obtain the binding affinity and interactive mode between ligand and receptor [29]. There are several small molecule databases, such as ZINC, PubChem, ChemDB, and DrugBank, which together surpass one million of deposited structures of potential ligands while 3D structure of proteins can be obtained from PDB or by computational modelling if it is not available [8-9-22-40-53-54]. There are several docking programs, such as DOCK , AUTODOCK [18-28], GOLD [25-50],and others. Each docking application is based on a specific search algorithm Each one has its specific parameter set and search method. The docking program taking into account several parameters, considering interatomic interactions, such as hydrogen bonds and hydrophobic contacts.

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Molecular dynamics (MD) simulations:

Molecular dynamics (MD) simulation is a powerful tool to investigate protein stability,
dynamics, and functions because simulations can be performed on time scales of the order of milliseconds using special hardware [31-42]. MD simulations mimic the physical motions of atoms in the protein molecule present in the actual environment. he atoms are allowed to interact for a certain period of time, which will help to compute their trajectory in and around the protein molecule. Molecular Mechanics force fields are the cornerstone of biomolecular simulations, being used to compute the potential energy of a system of particles. he role of solvent is very important in simulation to determine the internal motion of proteins at different temperatures, particularly below the glass transition temperature, since experimentally it may be sometimes difficult to capture the dynamics associated with the internal motion of proteins [19-51]. MD simulation routinely calculates the following quantities: RMSD, RMSF, Rg [20].

RMSD: It is one of the fundamental factors which is used to estimate protein stability compared to the initial structure. The RMSD trajectory is used to predict the stability of the protein. Higher RMSD value implies low stability of the protein structure and it measures the deviation of the atomic position and indicates any structural changes throughout the MD simulation. **RMSF:** The RMSF with respect to the average MD simulation conformation is used as a mean describing flexibility differences among residues. RMSF analysis is performed to evaluate the average fluctuations of residues during the simulation. Higher RMSF value shows more flexible movements whereas low RMSF value shows limited movements during simulation in relation to its average position. **Rg:** Rg, as a mass-weighted root mean square distance of a group of atoms from their common center, is used as a criterion of protein compactness. Therefore, Rg analysis indicates the overall dimensions of the protein. [36-39-41-43-52]

In recent years, some widely used MD simulation packages such as NAMD [35], GROMACS [37], and AMBER [6] have all substantially improved their algorithmic sophistication and parallel performance, being able to deliver up to ~ 10-100 ns/day/workstation/cluster [13]. MD simulation provides an alternate approach to the study of protein dynamics at NMR relaxation time scales [46].

Research Justifications

Wheat (*Triticum aestivum*) is among the most widely cultivated crops in the world. Spot blotch of wheat caused by the hemi-biotroph fungal phytopathogen *Bipolaris sorokiniana* is now becoming a serious threat to wheat cultivation. To control this pathogen, chemical fungicides are being used at large scales, which is not an environmentally friendly practice. So, there is a need to develop specific and environment-friendly methods for controlling which might be obtained by a proper and in-depth understanding of host–pathogen interactions at a molecular level.

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Research objectives

To decipher the molecular interaction between Gα subunit of wheat and the *Bipolaris sorokiniana* toxins for in-depth understanding of host–pathogen interactions in order to have better management of spot blotch disease in wheat and hence reduce yield loses.

II. MATERIALS AND METHODS

3D model generation of G-protein alpha subunit:

The 3D structures of G-protein alpha subunit from *Triticum aestivum*, proteins are not available in PDB. Thus, the amino acid sequence of G-protein alpha subunit was fetched from NCBI protein database (BAC10502.1). BLASTp search was executed against the PDB to find appropriate template protein structures for homology modelling. The best template (PDB ID: 2XTZ) showing maximum identity and high score as well as lower e-value was selected for building 3D structures.

The sequence alignment between target and template was performed using MultAlin program. MODELLER9v19 was used for 3D structure prediction. Out of 20 models (3D structures) resulted from MODELLER, one 3D structure was selected and subjected to further stereo-chemical properties check to get the deviations from the standard bond length, dihedrals and non-bonded atom-atom distances. The 3D structure models of G-protein alpha subunit were further calculated based on discrete optimized protein energy (DOPE) scores. The model with lowermost DOPE scores was selected for further refinement using GROMACSv5.1 simulation package.

Model validation

The stereo-chemical quality of generated 3D structure was verified using SAVES server, ProSA and ProQ. MolProbity tool was used to calculate bond length and bond angle of the 3D model. The stereo-chemical properties and Ramachandran plots was analysed using Procheck analysis. Furthermore, the Z-score, packing defects, bump score; radius of gyration and deviation of Y angles of the selected model was observed using VADAR, GeNMR and PROSESS web-servers. Additionally, to evaluate the accuracy of the model, the structural superimposition of corresponding Cα trace of the predicted structure over template structure (PDB ID: 2XTZ) was executed using PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.).

Molecular Docking

To obtain insights into the mode of binding of 'Prehelminthosporol (CID_6451296)', 'Helminthosporic acid (CID_14165721)', 'Sorokinianin (CID_10881395', and 'Bipolaroxin', we employed the molecular docking suite AutoDock4.2. Docking studies were performed on the active site of the modeled G-protein alpha from *Triticum aestivum*. Based on the structural superposition with the structural homologs, the ligand-binding residues were noted and compared with ligand-binding pocket identification tools HotSpot Wizard 3.0,

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CASTp, and GalaxySite (https://galaxy.seoklab.org/cgi-bin/submit.cgi? type=SITE). The structure of 'Prehelminthosporol', 'Helminthosporic acid', 'Sorokinianin', and 'Bipolaroxin' were obtained from PubChem and optimized using Automated Topology Builder (ATB) and Repository Version 3.0. The following parameter was employed for docking in AutoDock; Lamarckian genetic algorithm with the number of runs: 300; population size: 150; the maximum number of evaluations: 25,000,000; the number of generations; 27,000; and the rate of cross over: 0.8. AutoDock scoring function (i.e., short-range van der Waals and electrostatic interactions, hydrogen bonding, and entropy losses) were included for binding energy estimation. The grid was set based on the consensus list of residues covering the ligand-binding pocket in the x, y, and z-axis directions with a grid spacing of 0.375 Å. The RMS cluster tolerance was set to 2.0 Å. Based on the RMSD-based clustering approach, the ligand binding poses were ranked using higher negative binding free energy with a greater number of H-bonds. AutoDock generated the lowest binding energy (most negative) docking conformation of Gα, subunit-'Bipolaroxin', Gα, subunit-'Prehelminthosporol', Gα, subunit-'Helminthosporic acid', Gα, subunit- 'Sorokinianin' complex was then subjected to protein–ligand interaction visualization using LigPlot+ and BIOVIA Discovery Studio Visulizer4.5 (BIOVIA DSV)

Molecular dynamics (MD) simulations

To study the dynamical properties, MD simulations were performed for modelled G protein alpha subunit of Triticum aestivum through GROMACSV5.1 package. For topology building, CHARMm force fields were used in cubic boxes with TIP3P water model; periodic boundary conditions were applied by keeping the distance between the edge of the box and the protein as 1nm. The protonation states of all the ionisable amino acids were determined at pH 7.0. The structures were solvated and sodium counter ions (0.15 M NaCl) were added to neutralize the overall charge of the systems. Energy minimization of the solvated systems were performed using conjugate gradient and steepest descent algorithm until maximum force reached less than 1000 kJmol-1nm-1. After energy minimization, each system was subjected to NVT and NPT ensemble for equilibration at 1000 ps. LINear Constraint Solver (LINCS) algorithm was implied to constrain all bond lengths. Electrostatics was calculated by using Particle mesh Ewald method with a cut-off distance of 0.9 nm. Finally, the equilibrated systems were subjected to final MD run of 50 ns at 300 K.

Analysis of MD trajectories

The intrinsic stability parameters i.e., backbone root mean square deviation (RMSD), radius of gyration, solvent accessible surface area (SASA), inter-molecular Hydrogen-bond and from each trajectory was analysed using utility toolkits of GROMACS and 2D graphs were plotted using Grace (http://plasma-gate.weizmann.ac.il/Grace/). The interaction images were plotted using BIVIA DSV and PyMOL.

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III. RESULTS AND DISCUSSION

3-Dimensionl structure of G-alpha subunit from wheat

For modeling the G-alpha subunit structure, the NCBI GenBank: BAC10502.1 was chosen as the target sequence. BLASTp against PDB suggested three putative templates i.e., 2XTZ (identity of 74%), 3UMR (identity of 37%) and 6DDE (identity of 37%) which were subsequently used for modeling using the multi-template approach.

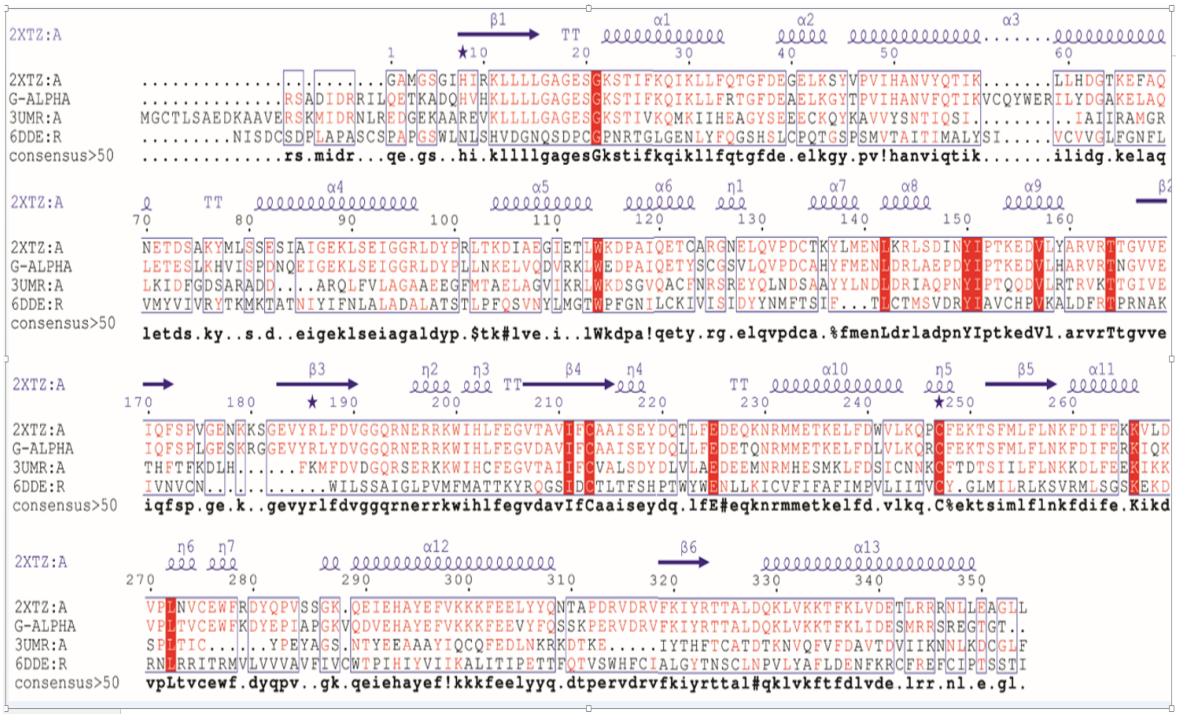

Fig. 1: sequence alignment of the three templates with the target G-alpha subunit from wheat

However, 2XTZ showed highest percentage of sequence identity with target sequence, but for better query coverage, we included the latter two templates for homology modelling. Due to lack of any structural information, the initial 20 residues were not considered for 3D modeling of G-alpha subunit from wheat. The sequence alignment of the three templates with the target G-alpha subunit from wheat has been displayed in Fig. 1. The model with the least DOPE score along with least Cα RMSD with the closest structural homolog 2XTZ was subjected to loop refinement and side chain optimization. Like the structural homolog, modelled subunit was found to be comprised of two domains i.e., the helical domain and Ras domain (Fig. 2A). Comparative analysis with the AtGPA1 (2XTZ) displayed minute differences in the variable loop region, however, the secondary structure elements were found to superpose well. Among the secondary structure

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elements, the beta strand comprised of 40 amino acids (10.8%), alpha helix composed of 165 amino acids (44.6%), 3-10 helix formed of 21 amino acids (5.7%) and turns/coils composed of 144 residues (38.9%). Overall, the structure was comprised of one beta sheet, 2 beta-alpha-beta units, 1 beta hairpin, 1-psi loop, 6 strands, 20 helices, 29 helix-helix interacs and 27 beta turns (Fig. 2B).

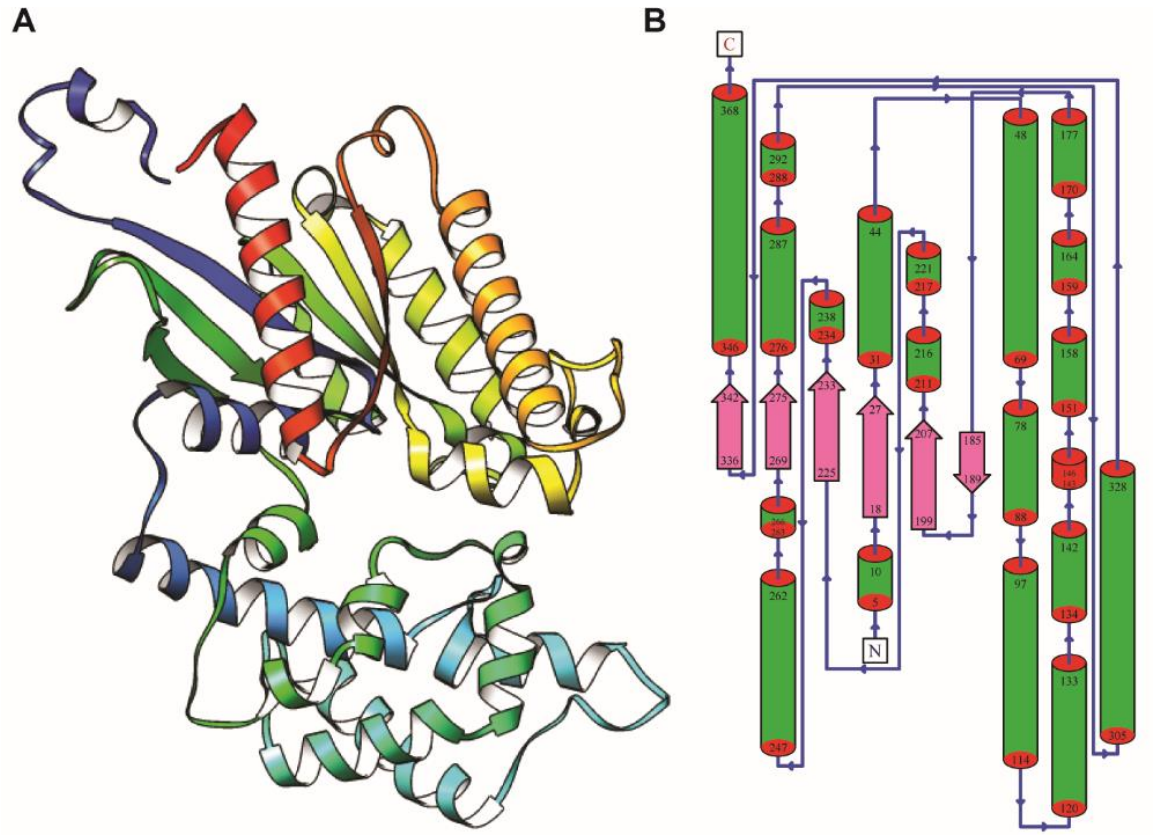

Fig. 2: Three-dimensional model and G-Alpha subunit. The solid ribbon representation of three-dimensional architecture of modeled G alpha subunit of wheat (A) with domains (helical and Ras domain).The topology of modeled the structure (B).

Validation of G-alpha subunit modeled structure

The overall stereo-chemical qualities of the model were evaluated to warrant that it was suitable for carrying out further studies. Prior to starting the actual evaluation process, the active site residues of the G-alpha subunit were determined by structural superposition with the coordinates of the backbone Cα atoms of the modeled structure and X-ray crystal structure of G alpha protein AtGPA1 from Arabidopsis thaliana (2XTZ-A chain) using PyMOL. It was observed that the active site residues of the 2XTZ crystal structure (bound to

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the 5'-Guanosine-Diphosphate-Monothiophosphate) found to superpose well with our modelled structure indicates that active site residues were found to be more or less conserved in G alpha sub units of plants. Stereo chemical quality of was explored using Ramachandran plot Procheck was employed to validate the rationality of homology model. Ramachandran plot of analysis displayed the dihedral angles Φ against Ψ of amino acid residues in the predicted model, where, 94% residues were found to be in the most favored region and none of the residues with bad geometry (outlier region of Φ and Ψ plot) depicts good quality of the model. ERRAT program was used to assess the overall quality factor of the modeled protein by determining the false statistics of bad non-bonded interactions within the structure. The overall quality factor of the modeled structure was 86.76% indicates the proposed model was of good quality. Additional analysis of the structure using the Profile-3D Verify score showed comparatively a good score of 82.22% indicating the correctness of homology model. Analysis of the predicted model using ProQ server indicated LGscore of "extremely good model" and MaxSub of "very good model" quality measures. ProSA-Web analysis also displayed the energy profile and Z-score of the model. Z-score for the predicted model was found to be within the range of scores typically found for the native proteins of similar size while the plot of residue energies revealed that entire calculated value was negative. Overall, the quality evaluation using various model validation servers suggested that the generated model is reliable (Table 1) and thus can be used for docking studies with higher confidence.

Table-1 Model validation of G protein alpha subunit of Triticum aestivum using various structural evaluation servers.

Model validation Servers	Model quality parameters	Validation scores
Procheck (Ramachandran plot)	Most favored regions (%) Additional allowed regions (%) Generously allowed regions (%) Disallowed regions (%)	94.0 5.1 0.9 0.0
Verify 3D	Averaged 3D-1D score >= 0.2(%)	86.76
Errat	Overall quality (%)	82.22
ProSA	Z score	-8.05
ProQ	LG score Max Sub	5.347 0.509
Prove	Z score mean	-0.041
METAMQAPII	GDT_TS	51.486

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Docking Analysis

Molecular docking was performed to understand the mode of 'Prehelminthosporol (CID_6451296)', 'Helminthosporic acid (CID_14165721)', 'Sorokinianin (CID_10881395)', and 'Bipolaroxin' binding in G-alpha subunit modeled structure. The resulted docking conformation from the docking experiment having lowest binding energy of -7,12, -9.23, -6.31, and -8.19 kcal/mol was selected as the representative structure for protein-ligand interaction studies respectively for 'Prehelminthosporol ', 'Helminthosporic acid', 'Sorokinianin, and 'Bipolaroxin' (Fig.3A, B, C and D). Like that of close structural homolog, the ligand prefers to bind prefer in the cavity formed of helical and Ras domain of G-alpha subunit where the residues of the helical domain participates in protein-ligand interaction. Protein-ligand interaction study using LigPlot+ revealed one H-bond formed by Lys302 and hydrophobic contacts formed by Tyr159, Gly162, Val167, Asp256, Gln257 and Ile298 with Prehelminthis, Protein-ligand interaction study revealed H-bond formed three H-bonds formed by Tyr159, Gly162, and Asp256 and hydrophobic interactions formed by Ser160, Cys161, Ser162, Ile298, Lys302 and Val306 with Helminthis. Protein-ligand interaction study revealed two H-bond formed by His172, Arg301 and hydrophobic interactions by Tyr159, Pro168, Asp169, Ile298 and Lys302 with sorokiana. Protein-ligand interaction study revealed H-bond formed six H-bonds mainly formed by Glu29, Ser30, Lys32, and Ala177. In addition to H-bonds, hydrophobic contacts formed by Gly28, Gly31, Ser33, Thr34, Arg78, Val179, Thr181 and Gly209 with Bipolaroxin were also observed (as displayed in Fig. 3A, B, C and D).

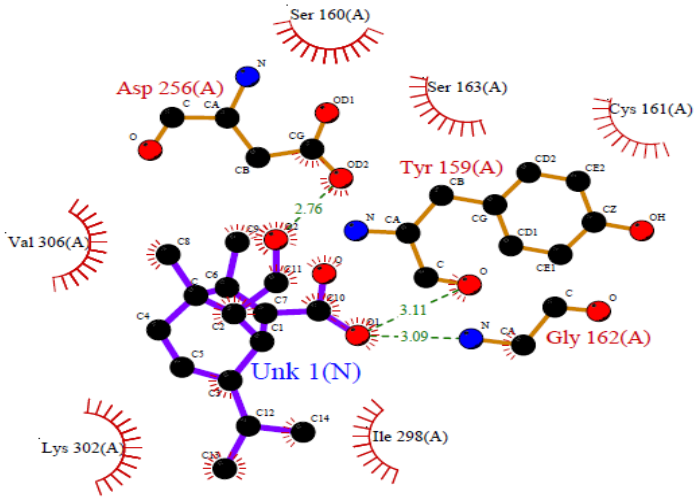

Fig.3A: The binding interactions between G-Alpha subunit and Prehelminthosporol

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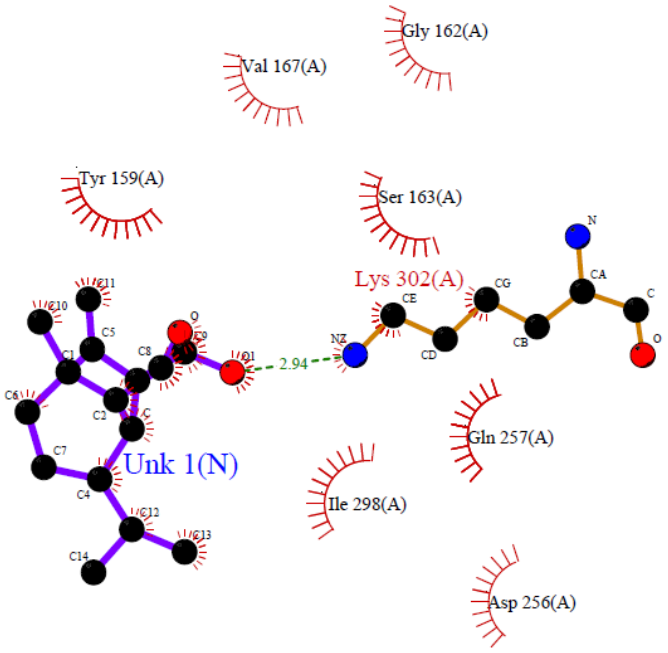

Fig.3B: The binding interactions between G-Alpha subunit and Helminthosporic acid

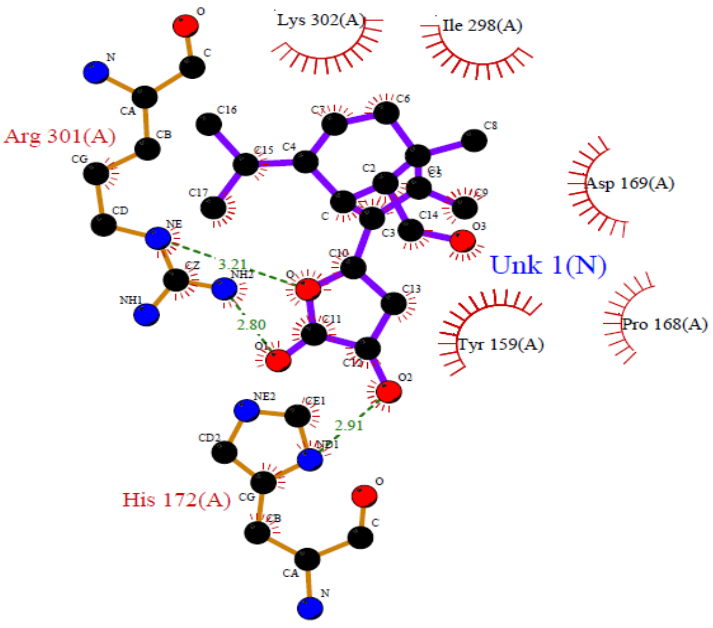

Fig.3C: The binding interactions between G-Alpha subunit and Sorokinianin

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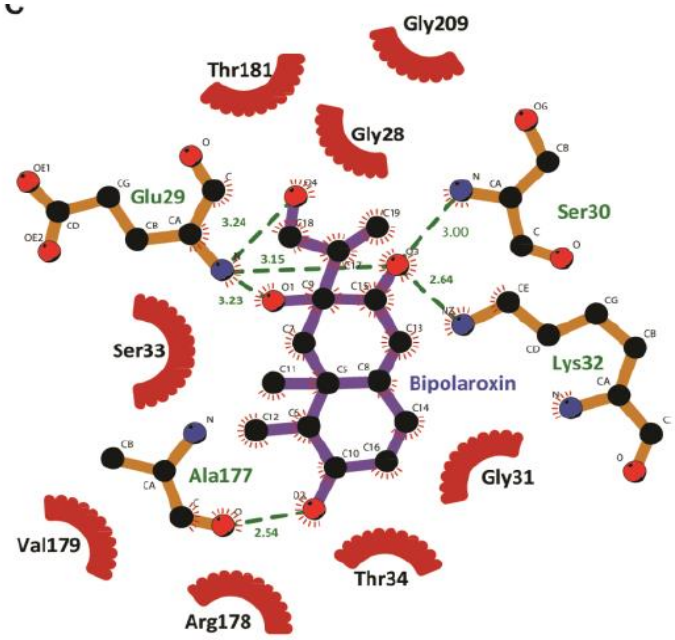

Fig.3D: The binding interactions between G-Alpha subunit and Bipolaroxin

Trajectory analysis

G protein alpha subunit of Triticum aestivum were submitted for MD simulations of 50ns in GROMACS. All systems were solvated in a cubic box. To gauge the intrinsic stability and dynamics of each system, the different stability parameters were calculated from each resultant trajectory. The backbone RMSD analysis provides important information on the stability of protein and protein-protein complexes and the time when simulation reached equlibrium. RMSD profiles of G protein alpha subunit of *Triticum aestivum* displayed least deviation with average RMSD ~0.25nm during the simulation period and by the time of 40 ns all both systems achieved convergence (Fig 4A). The residue flexibility of G protein alpha subunit was examined by performing Cα RMSF analysis. The protein displayed differential flexibility G protein alpha subunit protein gave a maximum value of 0.53 nm (Fig 4B). The RMSF values remarkably demonstrate the difference in residual flexibility in the proteins. Radius of gyration (Rg) represents the atomic distribution from their mutual center of mass in terms of mass-weighted root mean square distance. The Rg depicts the compactness and inclusive dimension of the protein and protein-protein complexes that may comprise their appropriate interactions. Statistical analysis of Rg for all the systems throughout the 50 ns trajectory showed distinctive difference (Fig 4C).

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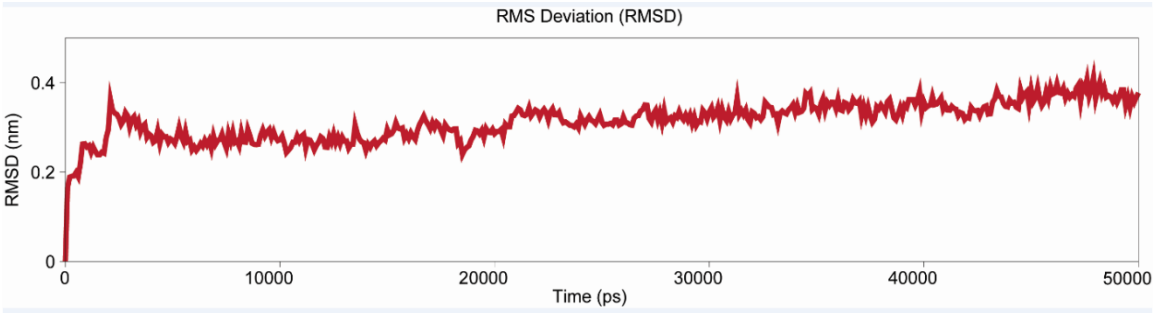

Fig-4(A) Root mean square deviation (RMSD) of backbone atoms of the modeled G-alpha sub-unit during 50 ns MD.

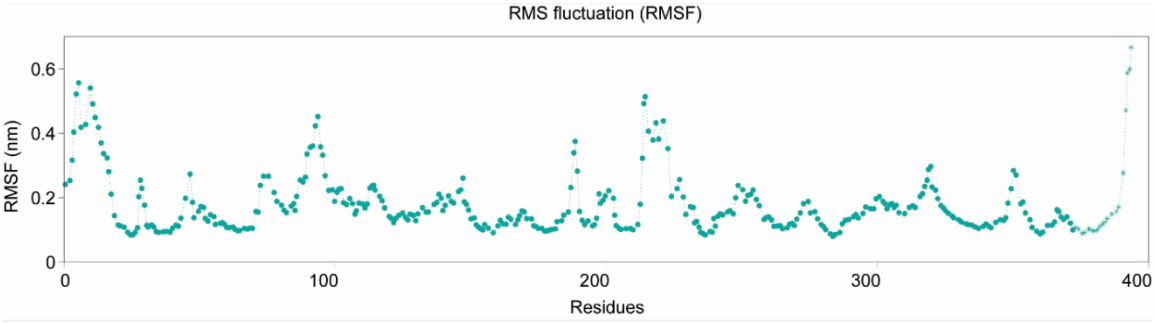

Fig-4. (B) The root mean square fluctuation (RMSF) for Cα atoms of the model.

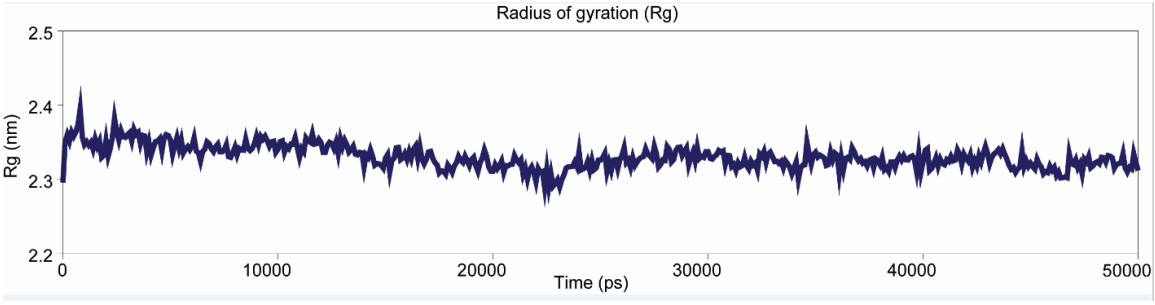

Fig-4 (C) The compactness of the trajectory by calculating the Radius of gyration (Rg) of the protein during 50 ns MD in aqueous solution

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IV. CONCLUSION

Bipolaris sorokiniana is considered one of the pathogenic fungi to wheat, causing the disease known as spot blotch, which is one of the most destructive plant diseases. It is characterized by the appearance of small dark brown spots, resulting from toxins and enzymes produced by the fungus during infection. This disease causes significant losses in wheat production in various countries worldwide, depending on different varieties.

Among the toxins produced by the fungus are helminthosporol, sorokinianin, prehelminthosporol, helminthosporic acid and bipolaroxin'. The interaction mechanism between these toxins and plant tissues has not been thoroughly studied yet. In this study we have tried to clarify the nature of the interaction and binding between these toxins produced by the fungus *Bipolaris sorokiniana* and the alpha subunit of G protein in wheat (*Triticum aestivum*) at a molecular level. This was done through modeling the 3D structure of the alpha subunit of the G protein in wheat and studying molecular docking between this subunit and those toxins followed by molecular dynamics (MD) simulations.

Recommendations and Future Perspectives

- This study represents a promising and fundamental step towards understanding the mechanism of interaction between the pathogen and the host plant.
- It is recommended to enhance this study with subsequent laboratory experiments that link the theoretical results obtained using bioinformatics tools with practical outcomes.
- Expanding the investigation to include a larger number of toxins with the subunits of G-protein is suggested.
- Exploring the possibility of genetic modification on the variable trimeric G-protein to confer resistance to the pathogen in plants is also recommended.

This could lead to the discovery of new protection and control mechanisms against the disease, ultimately improving disease management.

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