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Genomic and structural studies on S-Glutathione transferases to confer herbicide tolerance in rice through computational approach

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The usage of herbicides is essential to sustain agricultural productivity throughout the world. Plants have natural mechanisms to detoxify herbicides where the most important steps are catalyzed by certain enzymes such as cytochrome P450 mono-oxygenases (CYPs) (catalyze oxidation reactions) and glutathione transferases (GSTs) (conjugate electrophilic herbicides with the tripeptide glutathione (GSH). The GST/GSH system has been found to play a major role in the detoxification of several classes of herbicides and therefore in the herbicide-resistance mechanism of weeds. GST structures have been determined experimentally in *Zea mays, Arabidopsis thaliana, Glycine max*, *etc*. that provide structural and functional insights on xenobiotics detoxification on rice. There is a lot of unexplored space regarding the structure and ligand binding specificity of rice GSTs in plants. In this study, we have analyzed the rice GSTs of various classes and have modeled their structure to understand their conserved nature. It is important to understand the similarity and their interaction pattern with herbicides observed among the diverse class of GSTs expressed in the rice. Rice genomic data available in public databases were used to predict the conserved and variable regions in rice GSTs. Sequence-wise comparison of 79 GST sequences categorized all the seven GST classes separately and no similarity between them was observed. Structure comparison reported similarity between the Phi and Theta classes having the least RMSD value of 1.195Åwhereas the Dhar and Phi classes were highly diverging with the highest RMSD value of 3.051Å. The docking result showed the higher affinity of glutathione, and chlorsulfuron herbicide towards ZETA protein compared to other GST classes. Further alanine mutation analysis reported the higher binding efficiency upon mutating ARG 24 and TYR 102 amino acids towards the binding of chlorsulfuron herbicide, respectively. Molecular dynamics simulation using GROMACS was successful in capturing the dynamics of the alanine mutated Zeta-Chlorsulfuron complexes, and the stable behavior of the complex may have implications for the herbicide detoxification process.

Keywords: Chlorsulfuron, Detoxification, Glutathione-S-transferase, Herbicide, Rice

Rice (*Oryza sativa* L.) is one of the most widely consumed staple foods in the world. It is the most important cereal grain that belongs to the grass family. There are many different varieties of rice, each with its own unique characteristics and uses. Rice is grown in both wetland and upland environments, with countries like China, India, and Indonesia being the largest producers. It is one of the most important crops in the world, providing sustenance for billions of people[1,2]. However, its growth is often hindered by the presence of weeds, which compete for nutrients and sunlight. To combat this issue, farmers rely on herbicides, which are chemicals designed to kill or inhibit the growth of weeds.

Herbicides play a crucial role in detoxification, helping to eliminate harmful substances from our environment and promote healthy plant growth. They are used to control or kill unwanted plants, commonly known as weeds, in agricultural, residential, and industrial settings. For the purpose of preserving and boosting productivity, today's worldwide agricultural land terrain relies heavily on the cautious utilization of herbicides[3]. One of the key benefits of herbicides is their ability to target specific types of plants while leaving other desired plants unharmed. This selective action is achieved through the use of different active ingredients that target specific enzymes or metabolic pathways found only in certain plants[4]. This detoxification process depends on two important groups of enzymes—cytochrome P450 mono-oxygenases (CYPs) and glutathione transferases (GSTs)[5].

Glutathione S-transferases (GST) are the essential enzymes that fall under the category of xenobiotics. GSTs are an ancient and diverse group of enzyme classes that are notable for their function in phase II

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detoxification reactions in plants[6]. There is a large gene family that codes for GSTs. GSTs are multifunctional, functioning as cytosolic dimers that facilitate the conjugation of electrophilic substrates with glutathione enzymes, which is an essential phase in detoxification[7]. Due to the significant agronomic potential of these enzymes in terms of herbicide selectivity, tolerance, and environmental safety, in-depth studies of plant GSTs are necessary. It has been described how GSTs are involved in a variety of processes, including the post-translational glutathionylation of proteins, the modulation of cell signaling kinases, the formation and modulation of ion channels, and oxidation-reduction reactions. In addition to their role in the detoxification of herbicides, GSTs are also involved in the biosynthesis of hormones, the breakdown of TYR and peroxide, stress signaling proteins[8], nodule function, and non-catalytic flavonoid-binding proteins[9,10]. A total of 79 GST genes were identified in the rice genome through genome-wide analysis[11]. Sequence analysis and the arrangement of putative motifs suggested that rice GST gene family members may have a variety of functions. Some rice GST genes showed tissue/organ, and developmental stage-specific expression patterns, according to an analysis of microarray data. 20 abiotic stresses, 32 biotic stresses, 32 arsenate stresses, and at least 31 GST genes responded to auxin and cytokinin plant hormones, respectively. Many of the GST genes in rice were frequently regulated by abiotic and biotic stresses, hormones, and developmental processes[11]. In rice, the glutathione S-transferase (GST) enzyme super family catalyzes reactions, provides defense against biotic and abiotic stresses, and improves herbicide resistance by conjugating the tripeptide glutathione (GSH) with hydrophobic substances[7,12]. This transformation creates a conjugated compound that is more soluble and less toxic. From bacteria to people, GSTs are a diverse superfamily of catalytic proteins that are essential to a wide range of biological processes[13].

This study aims to tackle the knowledge gap on rice GSTs by investigating their structural characteristics and revealing their interactions with herbicides. The findings from structural comparisons have lots of potential for identifying rice GST classes that are particularly effective at assisting herbicide detoxification processes. The mutation study gives valuable insights into how amino acid mutations may affect the detoxification procedure and illuminate the underlying mechanisms of herbicide detoxification in the Zeta protein. The insights resulting from molecular *in silico* studies affirm that they provide useful data for the formulation of strategies aimed at enhancing herbicide resistance in rice crops, thereby significantly boosting agricultural productivity worldwide.

Materials and Methods

Molecular modeling and protein preparation

Protein sequences from seven distinctive GST classes—PHI, TAU, THETA, ZETA, TCHQD, DHAR and EF1G were accessed through a systematic search in the Rice Annotation Project Database[14] (RAP-DB) using locus IDs (Loc IDs). Rice GST (Glutathione S-Transferase) sequences were first aligned using the ClustalW[15] algorithm for the purpose of creating a phylogenetic tree. The alignment process helps in the identification of sequence similarities and differences. Using specialized software called FigTree[16], the alignment results were visualized and represented as a phylogenetic tree. The only one of these with a known three-dimensional structure was the TAU protein (UniProt ID— B6T033). A combination of approaches was used to model the other six proteins. Three proteins (PHI, ZETA and DHAR) were subjected to Swiss Model[17] homology modeling using templates that were structurally related in order to predict the three-dimensional structure of the proteins. The remaining three proteins THETA, TCHQD and EF1G were modeled using the I-TASSER[18] ab initio method, which relied on *de novo* structure prediction because there were no suitable homologous templates available. The study of structural variation and active site prediction was done using CastP[19] and PDBeFold[20].

Ligand preparation

From PubChem[21], the 3D structures of the ligands S-Hydroxymethyl Glutathione (GSH) and rice herbicide chlorsulfuron, were acquired. The Open Babel[22] software tool was used to convert these structures from SDF (Structure-Data File) format to PDB (Protein Data Bank) format for further analysis.

Molecular docking

All seven protein classes were docked using Auto Dock[23] tools with S-Hydroxymethyl Glutathione (GSH) to start the molecular docking process. The resulting complex then endured a second docking round with the herbicide chlorsulfuron.

Preparation of input files

The protein and ligand structures were both precisely formatted in PDB (Protein Data Bank) format

in the first step of preparation of the input files. This involved the addition of any missing atoms or bonds and also the removal of unnecessary water molecules.

Preparation of receptor grid map

A receptor grid map was generated to define the search domain for docking simulations. Thesize of the grid box was adjusted to fit the protein's active site Serine. During this stage, the grid map was created using Auto Grid and saved in the (.gpf) format.

Setting docking parameters and executing auto dock

Docking parameters configuration comprises setting the population size to 1000 and the number of genetic algorithm iterations to 100. The Lamarckian Genetic Algorithm (LGA) is used to explore the conformational space of the ligand once the input files have been created and the parameters have been established. The Auto Dock is then run to determine the best binding pose, and the results are saved in the (.dpf) format.

Analysis of docking result

In order to determine the most desirable binding conformation, the docking results were carefully examined, concentrating on the poses that scored the highest negative binding energy. This analysis identified the interactions that were energetically favorable, which enables to better understand the precise molecular arrangement and forces governing these protein-ligand complexes.

GROMACS simulation of molecular dynamics

The docked complex was simulated to better understand its molecular behavior and forecast molecular interactions.

System preparation and solvation

Using GROMACS[24] software, the Molecular Dynamics (MD) simulation of the GST protein and ligand complex was carried out. By removing the HETATM, the protein and ligand were saved separately from the complex file. For the purpose of optimizing the system geometry, protonation, and minimization procedures were applied to the systems and simulated using the GROMOS96 force field. Using the PRODRG[25] server, the essential molecular topology file parameters for the ligands could be obtained. The docked complex was neutralized by placing it in water cubic boxes with solvated molecules and ions. Ions were then added for neutralizing the systems.

Energy minimization and equilibration

After the system has been prepared, energy minimization was performed to eliminate any disturbing connections or overlapping atoms. This entails fine-tuning the system shape to get rid of undesirable connections or overlapping atoms and arrive at a stable initial simulation configuration. The system is equilibrated to stabilize it at the required temperature, pressure, and other conditions after energy minimization. To make sure that the system is stable before the production run, this equilibration involves two phases. Applying restraints to the ligand, such as NVT (constant number of particles, volume, and temperature), is the first stage of equilibration. The second stage of equilibration involves treating temperature coupling groups with NPT (constant number of particles, pressure, and temperature) simulations.

Molecular dynamics production and analysis

After the system has reached equilibrium, a production run was carried out for 100 ns to recreate the system's dynamics over a specified time frame. The analysis was performed using the trajectory file created during a production run of a molecular simulation.

Mutation

Using the Swiss-PDB Viewer[26], we manually introduced mutations by substituting the identified amino acid residues with alanine in order to evaluate the impact of these interactions. The mutated protein structures were then put through a molecular docking analysis with glutathione (GSH) and the herbicide chlorsulfuron. This docking analysis was performed using AutoDock program, which allowed for the evaluation of the intermolecular interactions and binding affinities between the ligands and the mutant residues. BIOVIA discovery studio visualizer 2020 (Accelrys Inc. San Diego, CA, United States) software was used to visualize and comprehend the docking results.

Results

Molecular modeling and structural validation

The protein sequences of rice GST were subjected to molecular modeling technique. Templates with a similarity score of 60% and above were selected for modeling, and the relevant parameters are summarized in (Table 1). This table includes the Locus ID for all seven rice GST classes, their respective target sequences,

template structures, and detailed similarity scores. Phylogenetic tree analysis (Fig. 1) clearly demonstrated the grouping of rice GST sequences from the same class, highlighting their distinct class-wise clustering. Active site estimation revealed that the Theta class exhibited the lowest active site volume (117.64Å³), while the Tau, Phi and Zeta classes displayed the highest active site volumes, measuring 2609.40Å³, 2043.73Å³, and 3167.53Å³, respectively (Table 2). Notably, structural analysis indicated that

Table 1— Comprehensive Molecular Modeling Information for Seven Classes of Glutathione S-Transferase (GST) Proteins

Protein class	Loc ID	Protein name	Software Used	Template information
PHI	Os03t0134900-01	Similar to Glutathione S-transferase GSTF14	Swiss Model	*Populustrimula*– 4RI6 Similarity %- 45.28 %
TAU	Os10t0528100-01	Similar to Glutathione S-transferase GST 42	Uniprot ID – B6T033	AlphaFold Identifier— AF-B6T033-F1
THETA	Os11t0588300-01	Similar to Glutathione transferaseAtGST 10	I-TASSER	C score = 0.37 Estimated TM score= 0.76±0.10 Estimated RMSD = 5.0±3.2Å
ZETA	Os11t0245100-01	Similar to Glutathione S-transferase 2.	Swiss Model	*Arabidopsis thaliana* – Similarity %- 60.09 %
DHAR	Os06t0232600-01	Similar to Dehydroascorbate reductase.	Swiss Model	*Populustrimula*- Similarity %- 66 %
TCHQD	Os04t0435500-02	Glutathione S-transferase, C-terminal-like domain containing protein	I-TASSER	C score = 0.80 Estimated TM score= 0.61±0.14 Estimated RMSD = 7.7±4.3Å
EF1G	Os02t0220600-02	Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma).	I-TASSER	C score = 2.99 Estimated TM score= 0.38±0.13 Estimated RMSD = 14.3±3.8Å

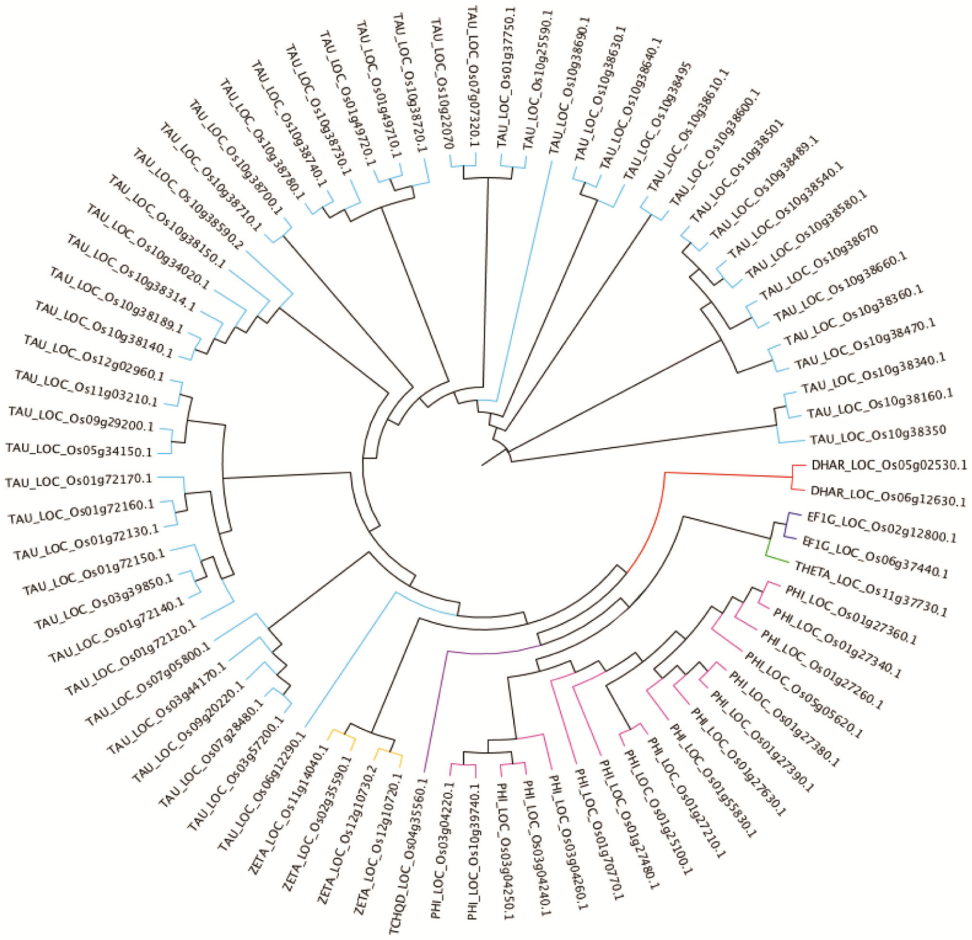
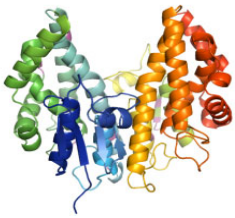
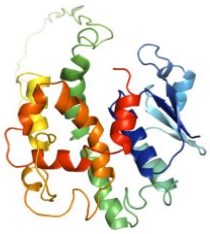
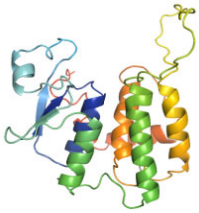
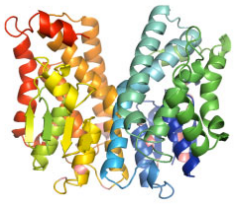
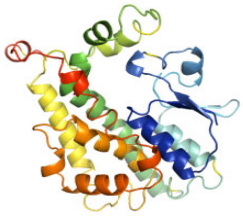
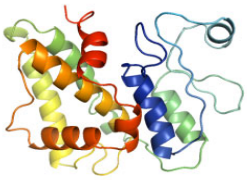
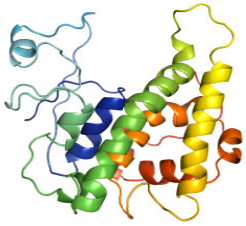


Fig. 1 — Phylogenetic tree showing the classification of GST proteins in rice

Table 2 — Modelled structures along with their active site prediction results

Classes	Structure	Active site surface area (Å²)	Active site volume (Å³)
Tau		1939.80	2609.40
Phi		1661.37	2043.73
Zeta		680.01	1119.02
Theta		269.46	117.64
TCHQD		981.334	553.80
Dhar		1070.56	547.28
EF1G		577.64	3167.53

the Phi and Theta classes shared the most similarity, with the lowest RMSD value of 1.195Å, whereas the Dhar and Phi classes exhibited the greatest structural divergence, marked by the highest RMSD value of 3.051Å. A comprehensive overview of RMSD profiles for all proteins is presented in (Table 3).

Molecular docking

The GH site (SER residue) was used as the active site in the molecular docking analysis to dock the GST proteins with the ligand glutathione. The herbicide chlorsulfuron was then docked to explore the underlying mechanisms of herbicide detoxification in plants. The results of the molecular docking analysis provide important novel insights into the stability of these herbicides' interactions with protein complexes. The resulting molecular binding energy are presented in (Table 4). Particularly, Zeta showed the lowest binding energies of -2.75, and -6.35 kcal/mol with glutathione and chlorsulfuron respectively, with higher number of hydrogen bonds compared to all other GST protein classes. Using Discovery Studio, these interactions were rendered and illustrated in (Fig. 2) which shows how glutathione interacts with the Zeta protein. From the docked result, particular amino acids, such as ARG 24, TYR 102, LEU 26, and ASP 82, were crucial in their interactions with glutathione which also influence the binding of chlorsulfuron.

Table 3 — RMSD values (in Å) by comparing the structures of the classes of GST

Structure	EF1G	Dhar	Theta	TCHQD	Tau	Zeta	Phi
EF1G		2.899	1.588	1.207	1.883	2.267	1.883
Dhar	2.899		2.901	3.007	2.624	2.918	3.051
Theta	1.588	2.901		1.381	1.996	2.237	1.195
TCHQD	1.207	3.007	1.381		1.765	2.291	1.772
Tau	1.883	2.624	1.996	1.765		2.383	2.250
Zeta	2.261	2.918	2.237	2.291	2.383		2.509
Phi	1.883	3.051	1.195	1.772	2.250	2.509	

Table 4 — Docking outcomes of glutathione, and chlorosulfuron with seven GST protein classes

Protein class	Glutathione		Chlorsulfuron	
	Best run	Binding Energy	Best run	Binding Energy
PHI	6	-1.62	77	-7.64
TAU	80	-2.75	77	-6.86
THETA	69	-1.31	36	-3.24
ZETA	51	-2.75	62	-6.35
DHAR	89	-2.34	42	-5.92
TCHQD	79	-1.69	62	-6.42
EF1G	42	-3.92	29	-6.85

Molecular dynamic simulation

For the Zeta-chlorsulfuron simulation (Fig. 3), the complex remained structurally stable, evidenced by consistent RMSD (Fig. 3A) fluctuations which got stable after an initial 5 ns while holding an RMSD value higher than 1.25 nm. Residues in the complex exhibited stable behavior within the range of 250 to 1250 and fluctuated with 0.1 nm as its minimum and 1.1 nm (Fig. 3B) as its maximum. The complex maintained compactness (Fig. 3C) with dynamic shape changes (Rgx, Rgy, Rgz overlap) and it is maintained stable at 1.70 nm after fluctuating from 2.3 nm to 1.75 nm. Hydrogen bonds (Fig. 3D) between Zeta and chlorsulfuron were observed throughout the simulation period indicating potential interactions. Solvent-accessible surface area (Fig. 3E)

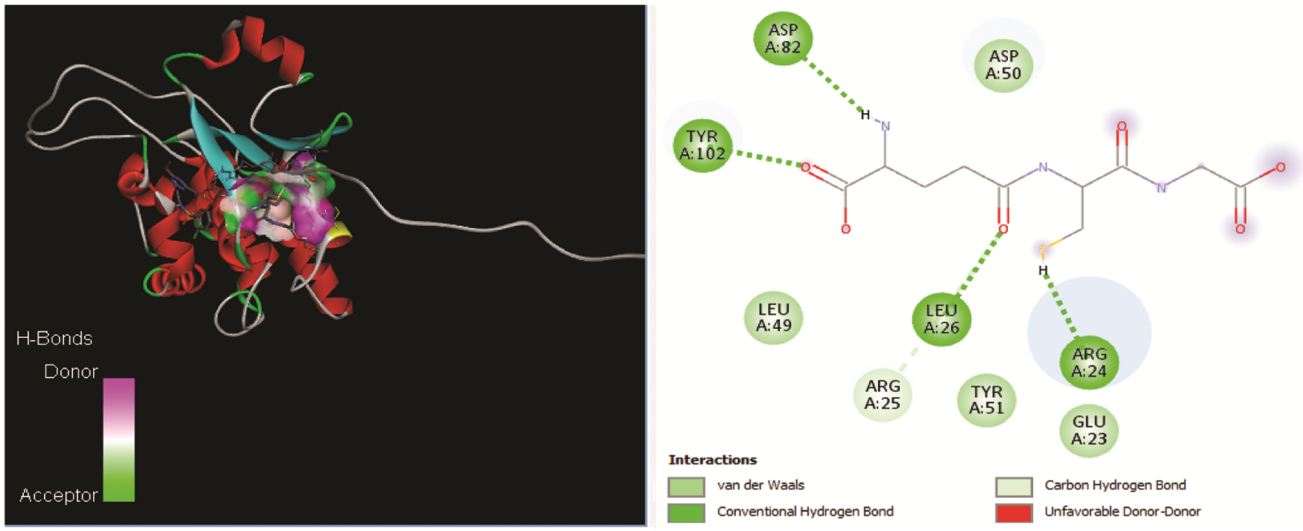


Fig. 2 — 3D and 2D interaction diagram of Zeta protein-glutathione complex

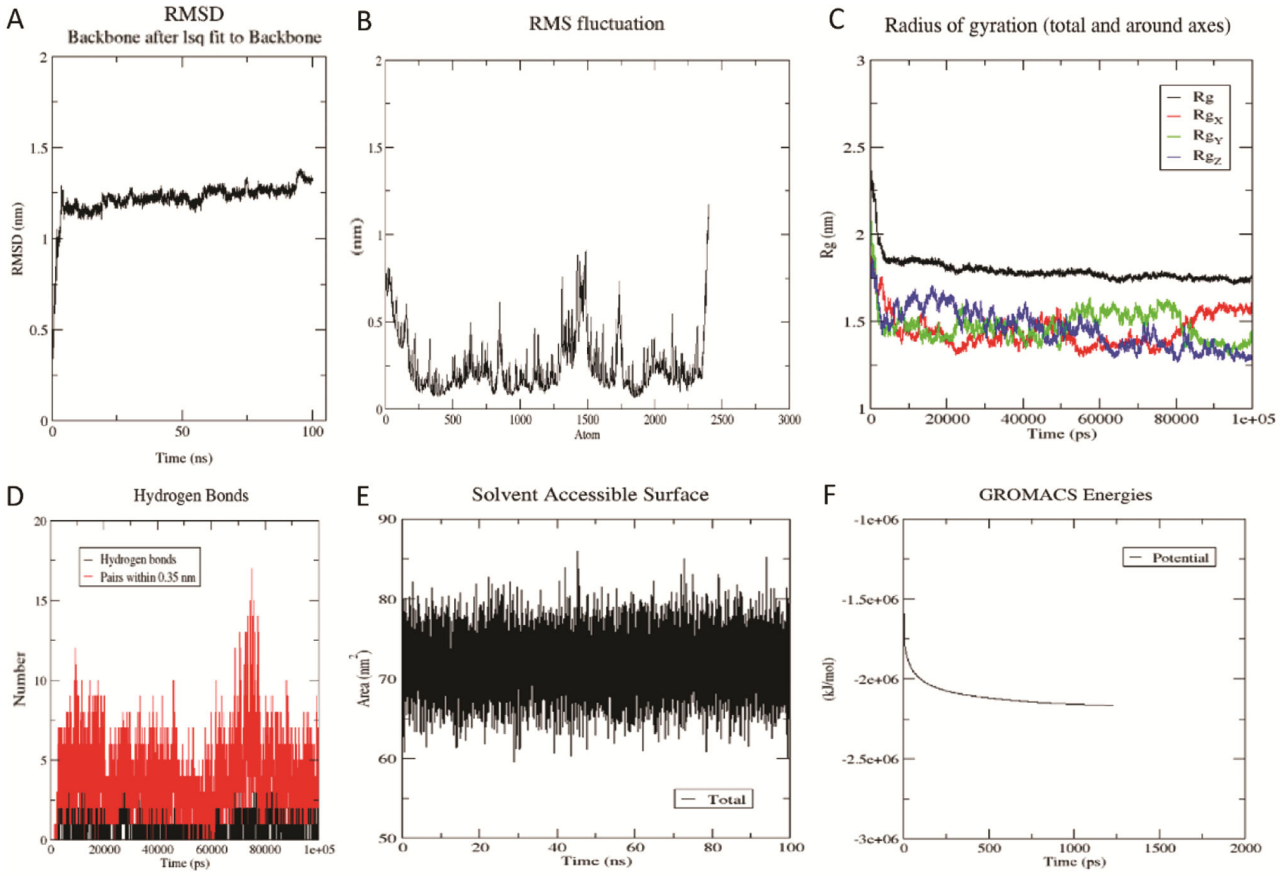


Fig. 3 — Zeta Protein (Wild type) with chlorsulfuron (Herbicide) in Molecular dynamic simulations. (A) Root Mean Square deviation (RMSD) of the complex explains the stability; (B) Root Mean square fluctuation (RMSF) of the complex; (C) Radius of Gyration; (D) Number of Hydrogen bonds formed; (E) SASA energy of the complex; and (F) Potential energy of the zeta-chlorsulfuron complex

Table 5— Docking results of the alanine mutated interacting residues

Residues	Glutathione		Chlorsulfuron	
	Best run	Binding Energy (Kcal/mol)	Best run	Binding Energy(Kcal/mol)
ARG 24	75	-3.47	83	-7.08
ASP 82	39	-2.90	49	-6.54
LEU 26	16	-2.66	21	-6.66
TYR 102	100	-3.04	72	-6.84

analysis showed consistent accessibility (60-80 nm²), enabling interactions with the environment. The potential energy (Fig. 3F) remained steady, confirming complex stability.

Mutation studies

The docking analysis showed considerably lower binding energy values in the mutated protein structure when compared to the wild-type protein after changing the interacting residues to Alanine. This indicates that the mutated protein and the herbicides have slightly stronger interactions. Table 5 shows the findings of the mutation study and the docking outcomes of the interacting residues with the alanine mutation. Illustrations are used to provide more insight into the interactions between the mutant protein and the herbicide chlorsulfuron (Fig. 4). In ARG 24 to ALA 24 mutation (Fig. 4A), glutathione docking reported hydrogen bond interactions with ASP 90, SER 91, SER 34, ARG 33, PRO 78, CYS 36, and VAL 77 amino acid residues with binding energy value of -3.47 kcal/mol. The mutation leads to the loss of the positively charged arginine, which may induce structural rearrangements that favor stronger binding of the herbicide chlorsulfuron compared to the wild-type. Whereas in ASP 82 to ALA 82 mutation (Fig. 4B), hydrogen bonds are maintained with GLN 130, ASP 90, SER 91, and SER 127, while a π-alkyl interaction with PHE 92 is present. The loss of aspartic acid's negatively charged carboxyl group alters electrostatic interactions within the binding pocket. However, the mutation appears to strengthen chlorsulfuron binding by promoting favorable structural modifications.

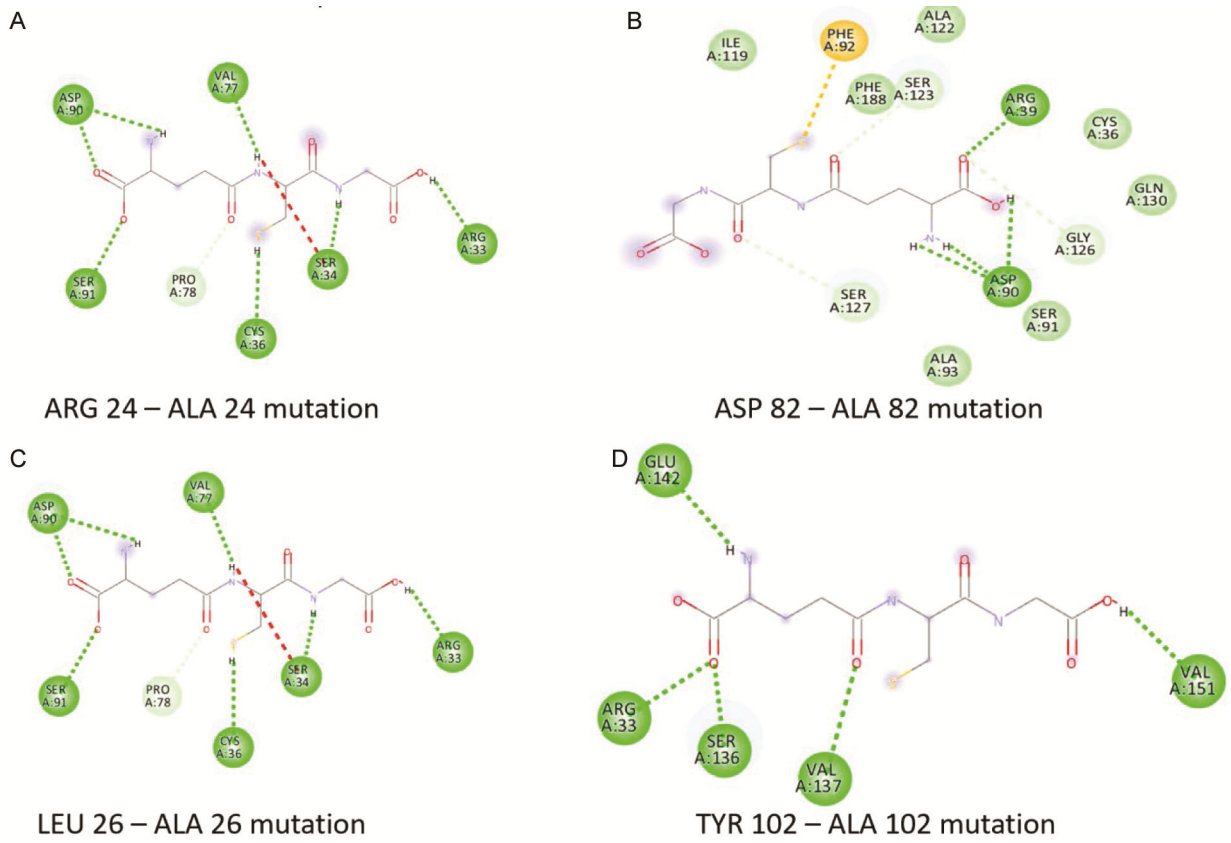


Fig. 4 —Interaction of alanine mutated Zeta Protein with -GSH Complex at binding site position of (A) ARG 24; (B) ASP 82; (C) LEU 26; and (D) TYR 102

Further, LEU 26 to ALA 26 mutation (Fig. 4C) reported the binding energy value of -2.66 Kcal/mol, hydrogen bonding occurs with ASP 90, SER 91, SER 34, ARG 33, PRO 78, CYS 36, and VAL 77 residues which stabilize the binding. In TYR 102 to ALA 102 mutation (binding energy of -3.05 Kcal/mol) (Fig. 4d), hydrogen bonds are formed with GLU 142, SER 136, ARG 33, VAL 137 and VAL 151 residues. The loss of tyrosine's hydroxyl group reduces direct hydrogen bonding potential but induces structural shifts that enhance chlorsulfuron binding. Finally, these mutations at the GSH binding site significantly increase chlorsulfuron binding affinity compared to the wild-type enzyme. In particular, the ARG 24 to ALA 24 mutation (Fig. 4A) removes a charged residue, likely reducing steric hindrance and improving ligand fit. This suggests that the mutation at the GSH binding site enhances ligand interaction by reducing steric hindrance and altering hydrogen bond networks.

In order to understand the role of ARG 24, TYR 102, LEU 26, and ASP 82 binding site residues in the binding of chlorsulfuron, MD simulation was carried out for 100 ns on a mutated complex where all the above mentioned four residues were mutated to ALA. Molecular dynamic simulations (Fig. 5A-F) in a mutated complex reveal that it attains a stable equilibrium after a few initial nanoseconds. Residues in the complex exhibited intricate behavior within the range of 50 to 2500 and fluctuated with 0.1 nm as its minimum and 1.0 nm as its maximum. The complex was compact with dynamic shape changes (Rgx, Rgy, Rgz overlap) and maintained stability. Hydrogen bonds were observed throughout the simulation period indicating potential interactions.

Discussion

A class of xenobiotic enzymes known as glutathione-S-transferases (GSTs) that are naturally occurring herbicide-tolerant are found in rice. GSTs are essential for reducing oxidative stress in plants, which significantly aids in a number of developmental processes. Through various putative genes, each of which directs various mechanisms, rice encodes these

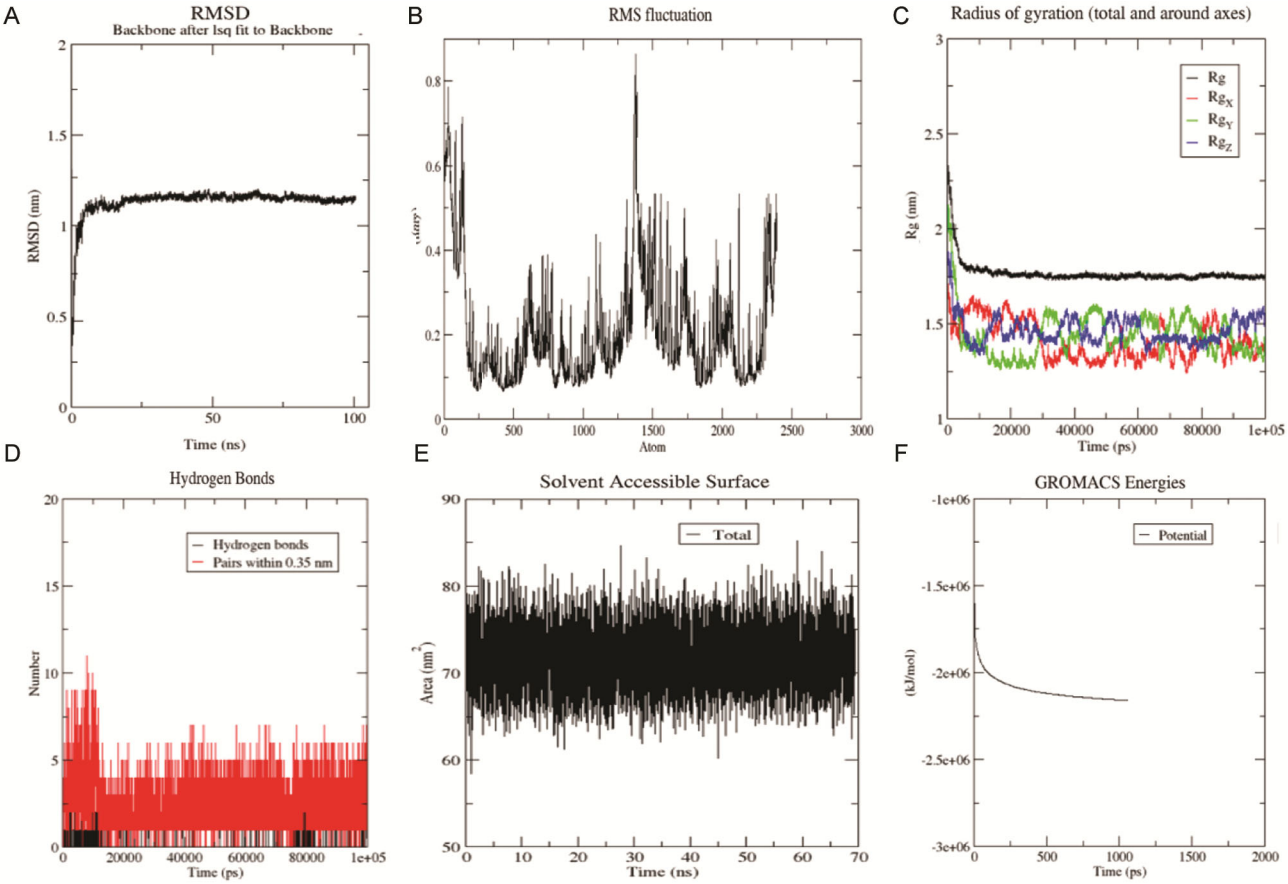


Fig. 5 — Zeta Protein (Mutated type) with Chlorosulfuron (Herbicide) in Molecular dynamic simulations. (A) Root Mean Square deviation (RMSD) of the complex explains the stability; (B) Root Mean square fluctuation (RMSF) of the complex; (C) Radius of Gyration; (D) Number of Hydrogen bonds formed; (E) SASA energy of the complex; and (F) Potential energy of the zeta-chlorsulfuron complex

GSTs. It has been found that the accumulation of OsGSTL1 protein in rice's vegetative tissues increases the plant's tolerance to chlorsulfuron. This emphasizes the crucial detoxification function OsGSTL1 performs in the growth and development of rice plants[27]. The over expressing OsGSTL2 gene at a higher level in transgenic rice showed its role in protecting the plants from herbicide injury[28]. In addition, GST plays a crucial role in cadmium stress response by enhancing detoxification in rice roots while being inhibited in shoots[29]. GST plays an important role in the detoxification of common agricultural contaminants glyphosate and chlorsulfuron. Numerous defense mechanisms like turning on resistance genes are active in the field of plant biology, and they are essential for reducing the toxicity of herbicides and promoting plant growth after they are applied[30-32].

A structural similarity analysis of the seven GST enzyme classes provided useful information for identifying potent xenobiotic proteins required for herbicide detoxification. The detoxification mechanisms of rice GSTs have been investigated through multiple molecular docking studies. The findings of this study provide insight into the molecular interactions of rice GSH enzymes with herbicides and highlight their function in recuperation processes. In particular, among all GST protein classes, the zeta class of enzymes indicated the lowest binding energies with chlorsulfuron (Table 4), highlighting their potential to control growth and trigger oxidative stress mechanisms in rice plants when exposed to various herbicides. Theta and Zeta classes of GST enzymes have glutathione peroxidase activity, which enables them to efficiently reduce the accumulation of cytotoxic hydroperoxides triggered by oxidative stress[33]. Srivastava *et al.* (2019)[34] revealed the role of Tau class GST proteins in controlling oxidative stress in rice, and this finding has been confirmed by molecular docking studies.

The illustrated phylogenetic tree of rice GSTs, as shown in (Fig. 1), provides information about the wide variety of GST proteins and their classification, which is primarily based on similarity scores. A comprehensive evolutionary tree analysis of rice GSTs, encompassing the majority of genes grouped into four different classes of plant-specific proteins, was revealed[35] in earlier research. With identity scores ranging from 17% to 98% when considering proteins within the same class, the Tau class, which has 40 genes, and the Phi class, which has 16 genes, are prominently featured in this classification.

Maia *et al* (2018)[35] reported the insecticide tolerant activity based trichloromethyl group of GSH enzyme in rice which confers the binding stability between the insecticides and enzymes. Also, earlier studies insisted the role of lysine and arginine, located at the catalytic sites of the GSH enzyme, have the potential to confer resistance or tolerance to herbicides in rice, particularly against substances like bentazon and metsulfuron[36]. GST protein constitutes an active site primarily composed of serine residues. Through the identification of binding pockets, the importance of ARG 24, TYR 102, LEU 26, and ASP 82 residues for binding glutathione and chlorsulfuron have been reported in this study (Fig. 2). The interaction of the above-mentioned residues underscores their role in confirming herbicide tolerance in rice when exposed to various herbicidal compounds. For mutation analysis, these four amino acids in the GSH catalytic site of the docked Zeta protein complex were replaced with alanine, the simplest amino acid. This study indicated the effect of mutation of specific residues in the binding pocket that can lead to enhanced herbicide binding, which may have implications for herbicide resistance or enzyme specificity modifications.

Conclusion

The study outlines the intricate world of rice glutathione S-transferases (GSTs) and demonstrates the structural variety, strong binding affinities, and detoxification mechanisms of these enzymes. It emphasizes the crucial function of GSTs as rice's naturally herbicide-tolerant enzymes, able to reduce oxidative stress and support essential developmental processes. The investigation also highlights the exceptional binding affinity of the Zeta class to herbicide chlorsulfuron and the distinct detoxification roles of various GST classes. The identification of essential amino acid residues necessary for herbicide interactions is another benefit of active site analysis. Studies on mutations highlight the manner in which wild-type GSH enzymes are less effective in binding herbicides than their alanine-mutated competitors. These results add to our understanding of rice's herbicide tolerance mechanisms and provide useful information for future agricultural endeavors and environmental protection.

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Conflict of interest

All authors declare no conflict of interest.

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