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Modeling and phylogeny analysis of bread wheat MnSOD

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Abstract:
Superoxide dismutase (SOD) acts as first line of defense against oxidative and genetic stress. Manganese superoxide dismutase (MnSOD), found in mitochondria or peroxisomes, contains Mn(III) at the active site. Therefore, it is of interest to study MnSOD from bread wheat (a grain crop). However, a structure model is not yet solved for bread wheat MnSOD. Hence, we describe the structure model of bread wheat MnSOD developed using homology model. The model provides molecular insight to metal binding molecular function towards the understanding of oxidative stress resistance in plants. The distinction of bread wheat (a monocot) MnSOD from dicots is also shown using phylogenetic analysis.

Keywords: MnSOD, monocots, dicots, structure model, stress

Background:
Wheat (*Triticum aestivum*) is the important grain crop of the family *Gramineae* (*Poaceae*) and the genus *Triticum.* Wheat, a polyploid (2n = 6x, AABBDD genomes), has a large genome of $16x10^9$ bp **[1]**. Abiotic stress such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agricultural productivity. Production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$), and the hydroxyl radical (OH•), during environmental stress, is one of the main reasons for decreases in productivity, injury, and death in plants. Plants have evolved very efficient antioxidant enzyme systems to scavenge ROS and also protects from injury caused by the oxidative stress **[2]**. Superoxide dismutase (SOD) is an important antioxidant enzyme protecting many cellular components by transforming superoxide anions (O_2^-) into H_2O_2 and O_2. Based on the metal cofactor the SOD enzyme is classified into three types Fe-SOD located in chloroplast, CuZn-SOD located in chloroplast and cytosol, Mn-SOD located mitochondria and peroxisomes, contains Mn(III) at the active site **[3]**. Manganese superoxide dismutase (MnSOD) is the primary antioxidant enzyme, widely distributed in prokaryotic and eukaryotic organisms and has been proven to be the only form of SOD essential for the survival of aerobic life **[4]**. MnSOD precursor protein encoded in the nucleus, is targeted to the mitochondrial matrix, and is processed to become the active MnSOD enzyme form after removal of the mitochondria-targeting leader sequence. The role of MnSOD in plants has been extensively studied owing to the unique property of mitochondrial protection and a possible role in tolerance of environmental stresses such as chilling, freezing, oxidative stress, aluminum toxicity, etc. **[5]**. The MnSOD holoenzyme in plants is composed of 4 subunits with a total molecular mass of around 91 kDa **[6]**. In bread wheat ($2n = 6x = 42$) mapping of 58 wheat ESTs revealed the presence of a total of 5 *MnSOD* genes on the long arms of the 2A, 2B, and 2D chromosomes hexaploid genome **[7]**.

There is increasing interest in the MnSOD genes in plants for enhancing tolerance of environmental stresses; however, there is insufficient information available on the gene structure and regulatory elements of the wheat *MnSOD* gene. A detailed analysis of the MnSOD sequences, their probable structures and mode of action is yet to be accomplished. In this study, MnSOD of *Triticum aestivum* was selected for which three dimensional structures were neither available at the protein data bank (PDB) nor at the ModBase database. Hence, we describe the structure model of MnSOD from bread wheat.

Methodology:
Template Selection:
The sequence of the wheat mitochondrial MnSOD was retrieved from SwissProt database with accession number Q56DH8. Template selection was done using BLASTp **[8]** for the query sequence against PDB (Protein Data Bank) database **[9]**. The target subsequently selected was the x-ray crystal structure with PDB code 1N0J with a resolution of 2.2 Å.

Sequence analysis:
All sequence alignments were completed using ClustalW **[10]**. ScanProsite **[11]** was used to identify consensus pattern.

Homology Modeling:
The 3D structure model of bread wheat MnSOD was development using MODELLER™ 9v8 **[12]**. Five models were built by model-output.py program of MODELLER™. The model with the lowest DOPE (Discrete Optimized Protein Energy) score was considered for further refinement and validation. Model was also generated by online modeling server, Swiss model **[13]** using same template.

Model Optimization:
The model was subjected for energy minimization. The minimization was carried out using GROMOS96 implemented incorporated in Swiss PDB Viewer [**14**]. The GROMOS96 helped in minimization of bond stretch energy of the modeled protein. It incorporated both bonded and non bonded form of energy occupied in the protein molecule.

Model Evaluation:
The model was evaluated on the basis of geometrical and stereo-chemical constraints using PROCHECK **[15]**, ProSA-Web **[16]** and Verify 3d **[17]**. RMSD (Root Mean Square Deviation) analysis of the predicted model from its template was calculated using SUPERPOSE **[18]**.

Prediction of Accessible Surface Area (ASA):
Accessible surface area of the model was predicted using ASAP server **[19]**.

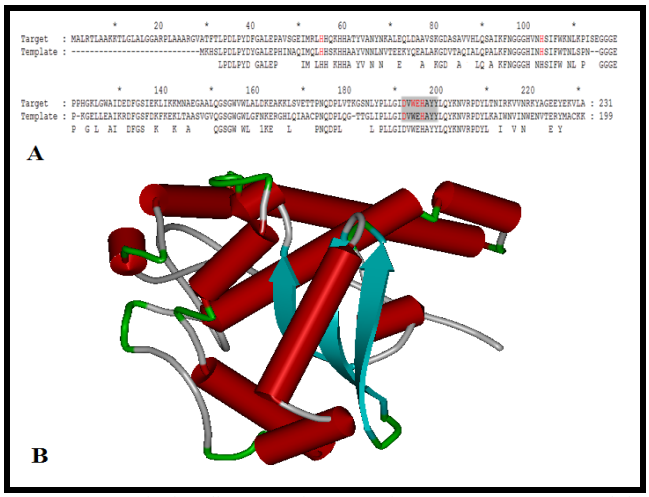

Figure 1: (A) Pairwise alignment of MnSOD from *Triticum aestivum* and the template (PDB ID: 1N0J). Dash represents insertion and deletion; conserved residues involved in metal binding are in red and shaded region showed the consensus pattern; (B) MnSOD structure model produced using Accelrys Discovery Studio *v2.5*.

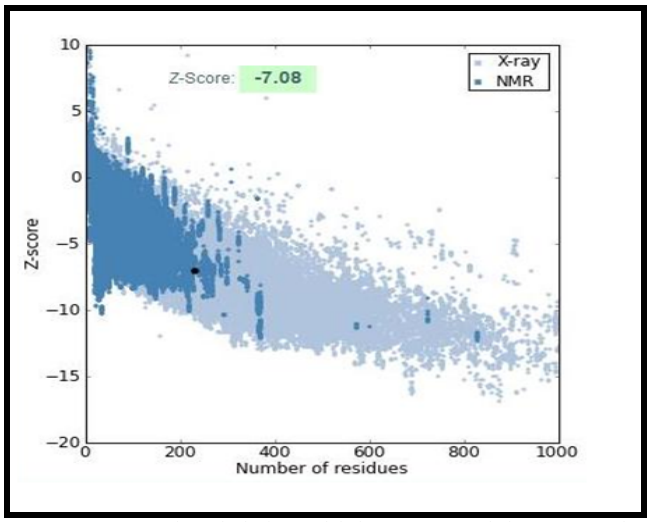

Figure 2: ProSA-web analysis for modeled MnSOD revealed a Z-score value of -7.08 (similar to the native conformations of the template).

Phylogenetic Analysis:
Protein sequences identical to bread wheat mitochondrial MnSOD were searched using NCBI BLAST program in *Glycine max, Hordeum vulgare, Oryza sativa, Zea mays* and *Arabidopsis thaliana*. Secondary structure prediction of wheat mitochondrial MnSOD using PROMALS3D was performed **[20]**. For evaluating the phylogenetic relationship, the selected sequences were aligned using alignment explorer in Mega 4.0 **[21]** with default parameters. Unrooted phylogenetic tree of 16 MnSOD sequences was constructed by the neighbor-joining (NJ) method in Mega 4 program. The level of confidence was estimated using bootstrap of 1000 replications.

Discussion:
The enzyme MnSOD is 231 amino acid residues long. Sequence search using BLAST identified the crystal structure with PDB ID: 1N0J. The human MnSOD (PDB ID: 1N0J) showed 58% sequence identity to the query sequence with an E-value of 1e-59. ScanProsite server identified the fragment DVWEHAYY from 192-199 residues as a consensus pattern. The sequence alignment revealed that the metal ion interacting residues His53, His103, Asp192 and His196 were conserved both in target and template **(Figure 1)**. We then developed structures for bread wheat MnSOD using MODELLER^{TM}. Five models were generated using this procedure. The model with the lowest DOPE (Discrete Optimized Protein Energy, a statistical potential used to assess homology models) score of -23108.04 was considered to be thermodynamically stable and chosen for further refinement and validation. The stereochemical quality and accuracy of the predicted model was evaluated using Ramachandran plot in Procheck. The MODELLER^{TM} generated model produced 91.5% residue falling in most favored region, 6.9% residues in additionally allowed region, 1.6% residues in generously allowed and with no residues in the disallowed region of the Ramachandran plot. This model is also compared with the Swiss Model with 90.0% residues in most favored region, 7.7% residues in additionally allowed region and 2.3% residues in generously allowed region. Hence, the structure produced using MODELLER^{TM} was used for further analysis.

ProSA-Web analysis of the model revealed a Z-score value (a measure of model quality as it measures the total energy of the structures) of -7.08 (negative value imply model accuracy) **(Figure 2)**. Verify3D showed 84.05% of the residues had a score greater than 0.2 for a good quality model. The degree of structure similarity is measured using RSMD (root-mean-square distance) between equivalent atom pairs. RSMD analysis of the MnSOD model was measured from its template using SuperPose. The Cα RMSD and backbone RSMD deviation for the model and the template crystal structure were 1.22Å, and 1.17Å respectively. Thus, the MODELLER^{TM} developed model was evaluated using several methods for reliability and accuracy.

Sequence and structural alignment of wheat mitochondrial MnSOD with its homologous sequences revealed that 8 helices and 3 strands were conserved in all the selected plant species **(Figure 3).** It showed high sequence similarity at the C-terminal with 3β sheets (β1-β3) and 3α helices (α6-α9) at the N-terminal. Accessible surface area prediction for MnSOD showed zero ASA values for metal ion interacting residue (His55, His103, Asp192, His196). Residues with ASA = 0 are buried and those with ASA > 0 are solvent exposed. Thus, the metal ion binding residues were buried and not accessible to solvent. Thus, the model provides molecular insight to metal binding molecular function towards the understanding of oxidative stress resistance in plants.

Phylogenetic analysis showed a clear demarcation of MnSODs into two large clusters. Cluster I comprised of sequences from monocot (*Hordeum vulgare, Oryza sativa, Zea mays* and *Triticum aestivum)* whereas cluster II included sequences from dicot (*Glycine max* and *Arabidopsis thaliana*) **(Figure 4)**. Each cluster has two subgroups; cluster Ia contained those from maize and rice, while cluster Ib have bread wheat. However, barley SOD clustered together with wheat indicated that wheat and barley are highly similar in comparison to rice, maize, soybean. In cluster IIa and IIb, soybean and *Arabidopsis* SODs form a distinct clade supported by highest bootstrap values. All the major clusters gave bootstrap values $\geq$ 60. The tree showed a distinct crop-specific clustering of sequences, representing clear crop specific sequence difference. The predicted model could be further explored for identification of ligand binding sites which may be useful to understand specific role of functional site residues during catalysis. Thus, the distinction of bread wheat (a monocot) MnSOD from dicots is shown using phylogenetic analysis.

Conclusion:
The molecular structure of bread wheat MnSOD is important for the understanding of its molecular function against oxidative stress. Superoxide dismutase (SOD) acts as defense against oxidative and genetic stress in plants. Hence, the structure model of bread wheat MnSOD developed using homology model is described. The model provides molecular insight to metal binding molecular function towards the understanding of oxidative stress resistance in plants. The distinction of bread wheat (a monocot) MnSOD from dicots is also shown using phylogenetic analysis.

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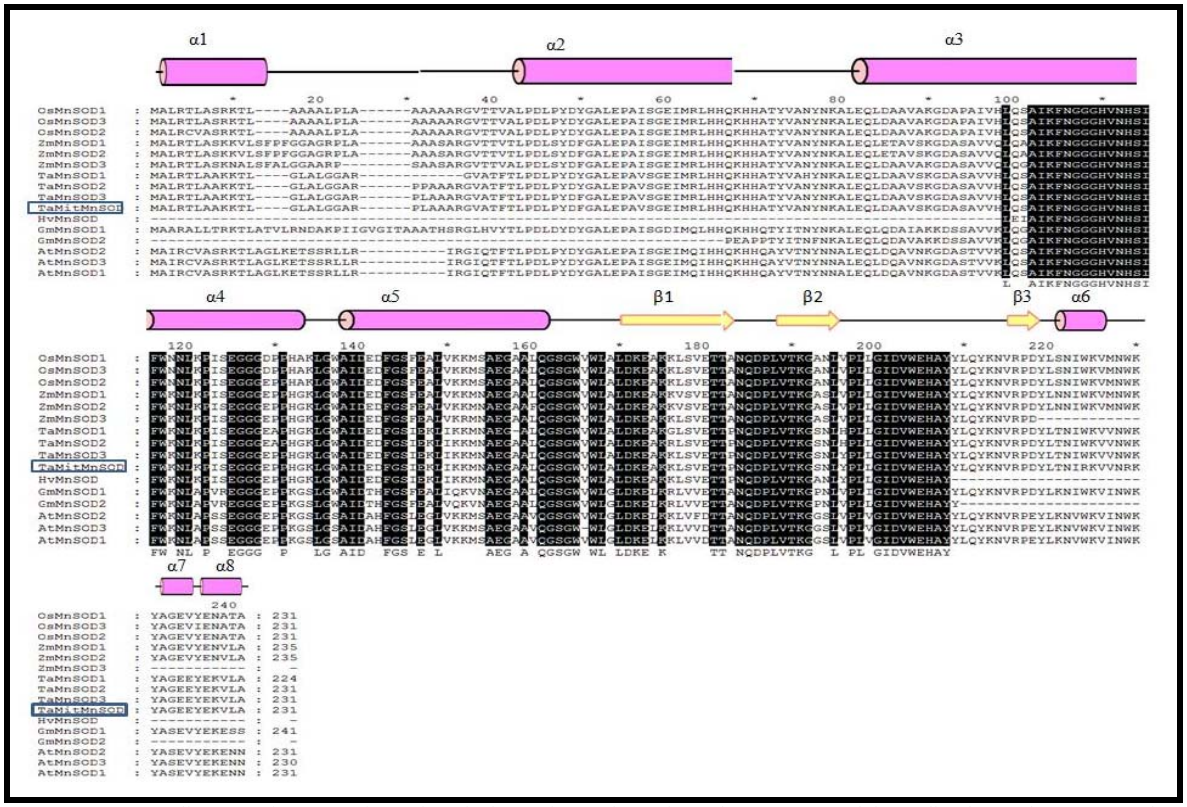

Figure 3: Sequence alignment of MnSODs from wheat, barley, maize, soybean, rice and *Arabidopsis* and the predicted secondary structure elements of the wheat mitochondrial MnSOD (TaMitMnSOD). Alpha helices and beta strands are represented as rods and arrows. Conserved residues in all plants are shown by shaded region.

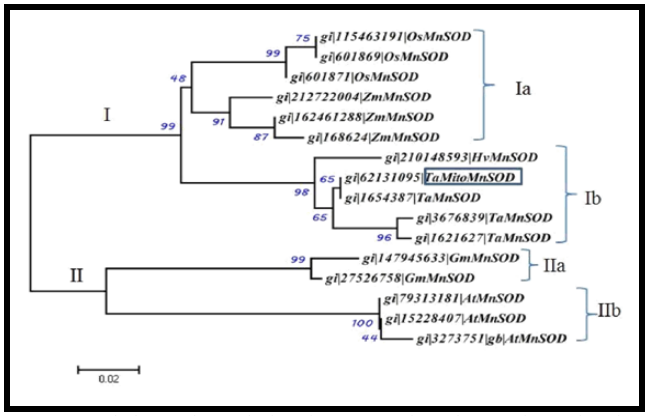

Figure 4: Phylogenetic analysis of *MnSODs* homologues from six plant species was constructed by the Neighbor-joining method using the MEGA 4 program. Bootstrap values are indicated against each branch. Phylogenetic analysis showed two large clusters of MnSODs. Cluster I comprised of sequences from monocot (*Hordeum vulgare, Oryza sativa, Zea mays* and *Triticum aestivum)* and cluster II included sequences from dicot (*Glycine max* and *Arabidopsis thaliana*).

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