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Proteome scale identification, classification and structural analysis of iron-binding proteins in bread wheat

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ABSTRACT

Bread wheat is one of the major staple foods of worldwide population and iron plays a significant role in growth and development of the plant. In this report, we are presenting the genome wide identification of iron-binding proteins in bread wheat. The wheat genome derived putative proteome was screened for identification of iron-binding sequence motifs. Out of 602 putative iron-binding proteins, 130 were able to produce reliable structural models by homology techniques and further analyzed for the presence of iron-binding structural motifs. The computationally identified proteins appear to bind to ferrous and ferric ions and showed diverse coordination geometries. Glu, His, Asp and Cys amino acid residues were found to be mostly involved in iron binding. We have classified these proteins on the basis of their localization in the different cellular compartments. The identified proteins were further classified into their protein folds, families and functional classes ranging from structure maintenance of cellular components, regulation of gene expression, post translational modification, membrane proteins, enzymes, signaling and storage proteins. This comprehensive report regarding structural iron binding proteome provides useful insights into the diversity of iron binding proteins of wheat plants and further utilized to study their roles in plant growth, development and physiology.

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1. Introduction

Bread wheat (*Triticum aestivum*) is one of the most important food crops and is a source of dietary protein, carbohydrates and minerals. It is one of the primary staple foods for the majority of global population [1]. After the data from the genome sequencing of bread wheat become available, there is vast opportunity to utilize the information and to understand the basic molecular mechanisms and physiology of wheat plant which may be proven further beneficial for the crop improvement programs [2,3].

Iron (Fe; atomic number: 26) is an essential metal ion for all the photosynthetic organisms and performs crucial role in normal growth and development of all the crops. Fe exists in two oxidation states, the bivalent (II) or ferrous (Fe^{2+}) and the trivalent (III) or ferric (Fe^{3+}). The Fe^{2+} and Fe^{3+} states of iron are inter-convertible and hence take part in reduction-oxidation reactions. In expanded oxidation states six and five electrons occupy the *d* subshell in case of the Fe^{2+} and Fe^{3+} respectively. In plant, bacterial and animal cells, iron cofactor with wide range of functionality may exist in the form of iron-sulfur (Fe-S) clusters, heme and di or mono nuclear iron. Fe-S clusters have an important role spanning from photosynthesis and respiration to epigenetics and DNA metabolism [4]. Iron is an important mineral micronutrient for humans and >2 billion people are suffering from deficiency of dietary iron [5]. Various attempts have been made to biofortify wheat with high grain iron content by using wide-hybridization which include, radiation breeding [6], induced homoeologous pairing [7] and other conventional molecular breeding techniques [8,9].

Plants follow two different strategies to uptake and transport iron *i.e.* strategy I (reduction based strategy followed by non-graminaceous plants) and strategy II (chelation based strategy followed by graminaceous plants) [10,11]. Previously, several experimental techniques have been utilized to investigate the metalloproteome from biological samples which included, high throughput tandem mass spectrometry, size exclusion chromatography linked with inductively coupled plasma atomic emission spectrometry [12], X-ray absorption spectroscopy [13], immobilized metal affinity chromatography and mass spectrometry [14] and metal isotope native radio autography in gel electrophoresis [15]. These analytical techniques are expensive, time consuming and need huge instrumentation infrastructure. These also have shown constrains in metal binding peptide identifications due to technical limitations in sample preparation, data processing and data acquisition [16]. Using experimental techniques, it was also

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identified that most of the metal binding proteins are uncharacterized in microbes despite advancement in instrumentation described above [17]. These reports further inferred that characterization of metal binding proteins are much more complex than previously thought and needed to be identified and classified using system scale and holistic approaches.

In this post genomic era, advancements in computational biology have opened new avenues to study the proteome of an organism. The bioinformatics tools may also be further utilized to scan the entire proteome to identify the iron-binding proteins and their functional annotation. Databases related to analysis of metal binding site in biological macromolecules have been established such as PROMISE (prosthetic centres and metal ions in protein active sites) [18], MDB (metalloprotein database and browser) [19], Metal-MACiE (metal-mechanism, annotation and classification in enzymes) [20], MetSite (protein-metal ion contact prediction) [21], MetalPDB [22] and Mespeus [23]. Similarly, various efficient bioinformatics tools have been developed to identify metal binding sites using protein sequences and structures such as CHED (Cys, His, Glu, Asp) [24], SeqCHED [25], FindGeo [26], MetalloPred [27,28], MIB (metal ion binding sites prediction server) [29], MetalDetector V2.0 [30], Metal S2 [31] and MetalS(3) (metal sites similarity search) [32]. By utilizing *in silico* approaches, iron-binding proteins were reported to localize in mitochondrion and cytoplasm in *Paracoccidioides* spp. This might be due to the fact that iron-binding proteins have an important role in energy production and electron transfer reactions at mitochondria in amino acid and carbohydrate metabolism in cytoplasm [33]. Genome wide analysis of metal binding proteins in human proteome using computational tools revealed ~22% proteins may bind to metals and ~14% of them may bind with transition metals including Fe [34].

Till date, there is limited information available on iron-binding proteins in bread wheat. Due to the significance of wheat as food for majority of global population and role of iron in human nutrition and plant growth, this study aimed at identifying and classifying the iron-binding proteins across wheat proteome. The detailed scheme for the identification and classification of iron-binding proteins is given in Fig. 1. In this report, out of 100,344 proteins of bread wheat, we have identified proteins carrying Fe binding sequential motifs. Out of those, on carrying out mass protein structure modelling 117 proteins showed to have high confidence Fe binding 3D motifs (novel as well as known). These results will further help in understanding the role of Fe binding proteins in wheat growth and development and could be beneficial in expediting the crop improvement program related to biofortification of wheat for high grain Fe content We have used sequence-based predictions to construct homology models of the putative iron binding proteins and studied the coordination chemistry of the binding sites using computational programs.

2. Materials and methods

2.1. Retrieval of proteome sequence dataset of Triticum aestivum

The whole proteome dataset of *Triticum aestivum* was downloaded from the file transfer protocol (FTP) servers of Ensembl Plants database (http://goo.gl/MLyVZt). The FTP "pep.all" dataset file was selected in this study which contained 100,344 protein sequences.

2.2. Protein sequence motifs search for identification of iron-binding proteins

All of the protein sequences were scanned with MetalloPred program to identify the putative iron-binding proteins [27]. MetalloPred identifies the main classes and subclasses of metal binding proteins using a three level cascade of neural networks from protein sequence derived features like amino acid and pseudo-amino acid composition and their physicochemical properties. The first layer of the cascade is to identify the metalloproteins, second layer is for the classification into alkali earth metal, alkali metal or transition metal and third layer is for the identification of the sub functional class of the bound metal. A total of 51 experimentally validated iron-binding reference proteins were selected from literature and sequences were retrieved from Protein Data Bank (PDB) to analyze them by Metallopred to optimize the threshold value (Fig. S-1). 0.951 was the lowest score obtained by any of the reference proteins in the set was selected as minimum threshold value for further analysis. This score was further utilized for screening of putative iron-binding sequence motifs from wheat proteome.

2.3. Mass homology modelling of putative iron-binding proteins

High throughput structure modelling of putative iron-binding proteins was carried out by means of the Phyre2 software program. Phyre2 uses detection method of advanced remote homology to build three dimensional protein structure models [35]. The modelled proteins with criterion - confidence score ≥ 90%, query coverage ≥ 50% and identity of ≥30% were selected. The quality of model was analyzed further with the similarity of sequence of the target and the template approximation. All the structure models were visually assessed for their intactness [36,37] and further validated by Ramachandran plot based VERIFY3D for any possible steric clashes [38,39].

2.4. Identification of iron ion binding 3D motifs

Metal ion-binding site prediction program (MIB) was used to computationally identify the Fe^{2+} & Fe^{3+} binding structural motifs from the obtained models [29]. MIB uses sequence and structural information to identify the iron ion binding residues around the centre of the metal ion. It aligns structurally to metal ion–binding residue template available in the database by fragment transformation method. An alignment score was assigned to each of the residues after comparing it with the template containing iron ions binding motifs.

2.5. Molecular interaction analysis within the binding sites

The protein structures containing iron-binding motifs obtained from MIB were visualized using Ligand Explorer (http://goo.gl/DxRVoP). It was used to analyze the interactions of bound ligands (Fe^{2+} & Fe^{3+}) in the protein structures. The options to display the network of interactions including metal interactions, hydrogen bonds, hydrophobic contacts and water mediated hydrogen bonds was used to view all metal binding interactions. After visualization in Ligand Explorer, the modelled structures were aligned with PDB template using PyMOL program [40] and protein structures carrying iron-binding sites with interaction radii up to 3.5 Å between residues and iron ions were further manually selected. The protein structures having binding sites consisting of iron ion interacting within 3.5 Å to 5 Å interaction radii considered as secondary shell and were also selected for analyzing the coordination geometry.

2.6. Assessment of coordination geometries

Coordination geometry of iron bound metal was analyzed using CheckMyMetal (CMM) program [41]. CMM evaluate the geometrical arrangement and inherent consistency of each metal binding site on the basis of several parameters including bond valence, metal binding sites, coordination numbers, allocation of sodium *versus* water and favourable isochemical environment for binding of metal ion in protein structures.

2.7. Sub-cellular localization and gene ontology analysis

A core set of total of 117 iron-binding proteins were predicted for their sub-cellular localization utilizing various bioinformatics tools

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