

Data mining of *Arabidopsis thaliana* salt-response proteins based on bioinformatics analysis

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Abstract

Salt stress limits the growth and productivity of many plants. To further understand plant protein salt-response mechanisms, the proteins of model plant *Arabidopsis thaliana* was selected and mined based on bioinformatic analysis and experimental biology verification. With the features of salt-response, 292 of 10,835 reviewed *Arabidopsis thaliana* proteins were screened from Uniprot public proteins database. Among 292 salt-response proteins, 67, 71, and 54 proteins showed the cross-talk with cold, drought and heavy metal stress, respectively. Function classifications revealed the above four groups (292, 67, 71, and 54 proteins) were mainly associated with reactive oxygen species scavenging and defense, catalytic activity, signal transduction, and energy metabolism. Activities of superoxide dismutase and peroxidase in both *Arabidopsis thaliana* root and leaf were determined under salt (150mmol.L⁻¹ NaCl), cold (10°C), drought (200mmol.L⁻¹ mannitol), and heavy metal (10µmol.L⁻¹ CdCl₂) stress. The experimental results were consistent with bioinformatic analysis. This work improves our knowledge of salt tolerance and provides clues to discover salt tolerance protein targets for plant growth and crop productivity.

Keywords: *Arabidopsis thaliana*; Bioinformatics; Cross-talk; Salt stress; Cold stress; Drought stress; Heavy Metal stress

Abbreviations: ROS- Reactive Oxygen Species, SOD-Superoxide Dismutase, POD-peroxidase, MS-Murashige and Skoog

Introduction

Soil salinity is a major environmental stress, which threatens global food security. Up to 20% of the world's irrigated land, which produces one third of the world's food, is salt affected (Alicia et al., 2009). Salinity decreases plant water uptake and mineral nutrition, and then causes ion imbalance, hyperosmotic stress and oxidative damage in plant (Zhu, 2002). Salt stress makes lots of plants slow growth, wilting or death (Munns, 2002). Therefore plant regulates the expression of salt-response proteins to reestablish cellular balance to reduce or adapt salt stress. As a result, plant changed in development, morphology, physiology and biochemistry (Kamal et al., 2010). *Arabidopsis thaliana* was used as an excellent experimental model to explore the mechanism of plant response and tolerance to salt stress (Munns and Tester, 2008). Many works have been done based on transcriptome and proteome techniques (Cui et al., 2010; Chan et al., 2010; Novillo et al., 2012). Transcriptome has extended to our understanding of salinity stress. Transcriptional profiling of NaCl-stress *Arabidopsis thaliana* roots was analyzed to reveal novel classes of responsive genes (Jiang and Deyholos, 2006). The results indicated that more than 20% genes of the genome changed, such as transcription factors, kinases/phosphatases, hormone-related genes. Those showed the complexity of plant salt response. But mRNA and protein levels are not always in agreement due to their post-transcriptional regulations (Gygi et al., 1999). In comparison with transcriptome, investigation of changes in plant proteome is more important since proteins, unlike transcripts, are direct effectors under salt stress. That

reason motivated us to explain plant salt response mechanisms at the proteome level. NaCl stress-responsive proteins in *Arabidopsis* roots have been analyzed by comparative proteome technique. 86 of the differentially proteins were identified, which were related to processes including scavenging for reactive oxygen species, signal transduction, translation, cell wall, biosynthesis, protein translation, processing and degradation, metabolism of energy, amino acids, and hormones (Pang et al., 2010). There are still two limitations: one is that most of these data are not publicly available, and the other is that any one published paper only provides limit data. Therefore enough data are essential to discover important plant salt response mechanisms. In this work, we screened 10,835 reviewed *Arabidopsis thaliana* proteins from Uniprot public proteins database to explore salt response proteins using the method of bioinformatic analysis and experimental biology verification. This investigation has improved our knowledge of salt tolerance in plant.

Results

Summary of *Arabidopsis thaliana* salt-response proteins

A total of 10,835 salt-response proteins were reviewed in the Uniprot database (Release 2011_11), and 292 proteins have the characteristics of salt-response (Supplementary Table 1).

Functional enrichment of 292 *Arabidopsis thaliana* salt-response proteins

For an overall overview of 292 *Arabidopsis thaliana* salt-response proteins, molecular functions were performed based on Uniprot database annotation. The results revealed that the largest number of proteins was related to catalytic activity (18.5%), the next prevalent were related to signal transduction (17.5%), the third were related to binding activity (12.0%), and the fourth were related to ROS scavenging and defense (11.6%) (Fig. 1, Supplementary Table 2).

Cross-talk: *Arabidopsis thaliana* salt-response proteins involved in cold, drought, and heavy metal stress

146 of 292 *Arabidopsis thaliana* salt-response proteins were also involved in the response of cold, drought, and heavy metal stress (Fig. 2, Supplementary Table 3). Among them, 67 proteins responded to cold stress (Supplementary Table 4); 71 proteins responded to drought stress (Supplementary Table 5); and 54 proteins responded to heavy metal stress (Supplementary Table 6). In 67 cross-talk proteins between salt and cold stress, the largest number of proteins was related to signal transduction (16.4%), the next prevalent were related to catalytic activity (16.4%), the third were related to ROS scavenging and defense (14.9%). In 71 cross-talk proteins between salt and drought stress, the largest number of proteins was related to signal transduction (19.7%), the next prevalent were related to catalytic activity (15.5%), the third were related to ROS scavenging and defense (12.7%). In 54 cross-talk proteins between salt and heavy metal stress, the largest number of proteins was related to ROS scavenging and defense (18.5%), the next prevalent were related to catalytic activity (14.8%), the third were related to energy metabolism (13.0%) (Fig. 3).

Arabidopsis thaliana growth and antioxidative enzymes activities under salt, cold, drought, and heavy metal stress

Under salt, cold, drought, and heavy metal stress, the *Arabidopsis thaliana* growth were all inhibited but with some differences. Under cold stress, root growth was inhibited but leaves were still green. Under drought, salt, and heavy metal stress, the root growth was inhibited and leaves were browning. Activities of POD and SOD in root and leaf were all significantly increased in comparison with control under four stresses. Additionally, two enzymes activities in root were all lower than in leaf. From cold, drought, salt, and heavy metal stress, two enzymes activities showed the trend of increase (Fig. 4).

Discussion

Arabidopsis thaliana is an excellent model with rich genetic resources for modern plant biology research. Although it is a true glycophyte, but one consistent theme was that salt-sensitive plants also had salt tolerance genes (Hasegawa et al., 2000). Proteome researches of *Arabidopsis thaliana* salt-tolerance mechanisms have been performed, but only identified some proteins with limited amount in the past years. To overcome the shortcoming, this study used 10,835 reviewed *Arabidopsis thaliana* proteins from Uniprot public proteins database to screen the salt-response molecules. UniProt database is a high quality database that serves as a stable, comprehensive, fully classified, richly and accurately annotated protein sequence knowledgebase. The UniProtKB

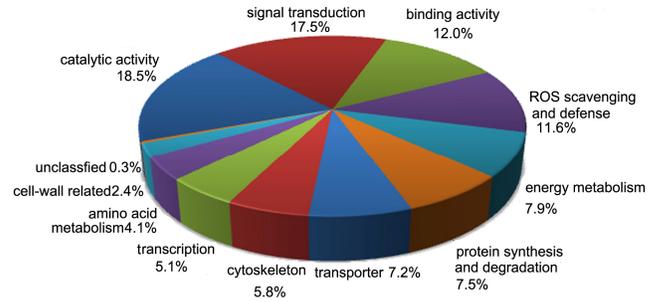


Fig 1. Pie diagrams of the proportion of 292 *Arabidopsis thaliana* salt-response by molecular function.

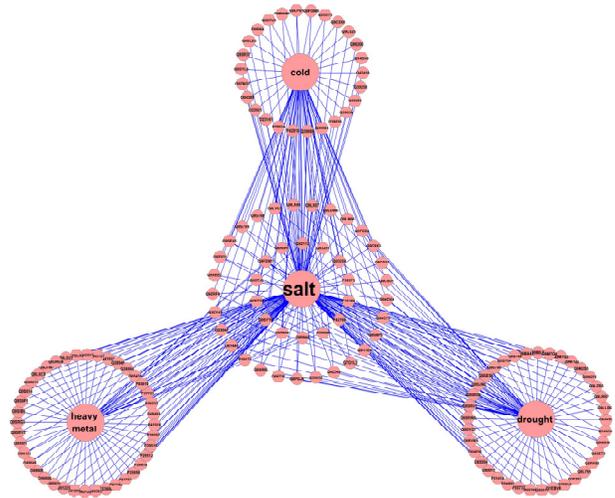


Fig 2. Cross-talk of *Arabidopsis thaliana* salt-response proteins with cold, drought, and heavy metal stress (54 salt-response proteins crosstalking with heavy metal stress; 71 salt-response proteins crosstalking with drought stress; 67 salt-response proteins crosstalking with cold stress).

GO annotation program aims to provide high-quality Gene Ontology (GO) annotations to proteins in the UniProt Knowledgebase (UniProtKB). The assignment of GO terms to UniProt records is an integral part of UniProt biocuration. UniProt manual and electronic GO annotations are supplemented with manual annotations supplied by external collaborating GO Consortium groups, to ensure a comprehensive GO annotation dataset is supplied to users. Its subsection of GOTERM_BP_FAT section provides information on the proteins response to various stresses. Its subsection of GOTERM_MF_FAT section provides information on the molecular function. These salient features of protein profiles is the compilation of up to date information, based on the available data in literature, which has been interpreted and described in the words of original researches (Schneider et al., 2004). In the present study, we screened 292 reviewed proteins response to salt in *Arabidopsis thaliana*, which exceeded the number of the traditional two-dimensional gel electrophoresis proteomics. This study provided us another angle to explore useful

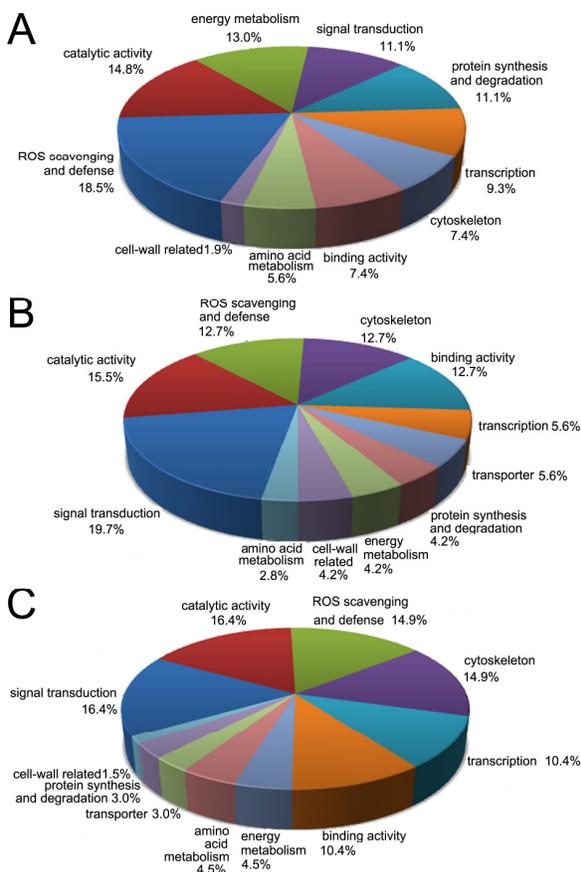


Fig 3. Molecular functional classification of *Arabidopsis thaliana* salt-response proteins crosstalking with other stresses (A: molecular functional classification of 54 salt-response proteins crosstalking with heavy metal stress; B: molecular functional classification of 71 salt-response proteins crosstalking with drought stress; C: molecular functional classification of 67 salt-response proteins crosstalking with cold stress).

information from large amount existing but often ignored database. Further bioinformatics analysis indicated the salt-response proteins had cross-talk with cold, drought, and heavy metal stress. In general, most drought-inducible genes are also induced by high-salinity stress, and many drought-inducible genes are also induced by abscisic acid (Shinzaki et al., 2003). Our results extended our understanding on plant proteins' cross-talk among various stresses. Function classifications revealed the above four groups (292, 67, 71, and 54 proteins) were mainly associated with ROS scavenging and defense, catalytic activity, signal transduction, and energy metabolism. In plant, antioxidative defense played an important role in adapting various stress (Abogadallah, 2010; Kosova et al., 2011). Base on bioinformatic analysis, two antioxidative enzymes were founded: POD and SOD. POD responded to salt-, cold-, drought, and heavy metal-stress, but SOD only responded to salt-, and heavy metal-stress. In consideration of protein cross-talk among different stresses, the results of experimental biology verification showed SOD also responded to cold and drought stress. That is to say, salt-response proteins might become potential biomarkers for other stresses, and other stresses-response proteins might also participate in salt stress. That indicated that the plant might

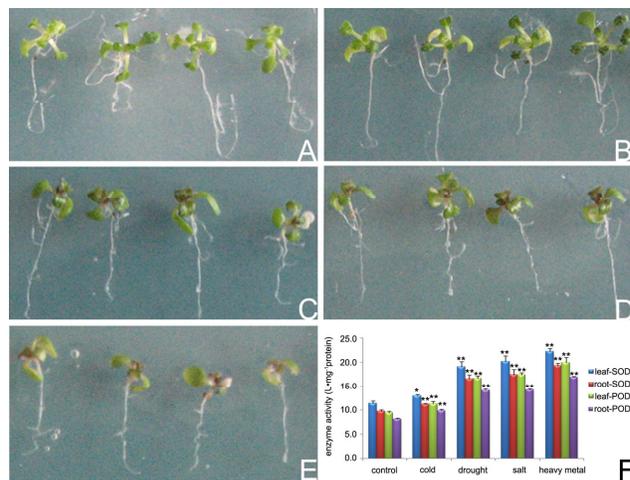


Fig 4. *Arabidopsis thaliana* growth and antioxidative enzymes activities under different stressed (A: control growth; B: cold stress with 10 °C; C: drought stress with 200mmol.L⁻¹ mannitol; D: salt stress with 150mmol.L⁻¹ NaCl; E: heavy metal stress with 10µmol.L⁻¹ CdCl₂; F: SOD and POD activities of *Arabidopsis thaliana* leaves and roots under cold, drought, salt, and heavy metal stress. The data represent the mean±SD obtained from at least three independent experiments *, $p < 0.05$; **, $p < 0.01$).

deal with various stresses in an economical way. In conclusion, this work provided new insight into *Arabidopsis thaliana* salt-response mechanism, and could urge scientists in this field to integrate all the existing data to explore salt-tolerance mechanism in plant. Additionally, *Arabidopsis thaliana* salt-response proteins had cross-talk with other stresses, which informed us to analyze the nature of salt-resistance with a wider field. Experimental biology verification is warranted to substantiate the bioinformatic analysis, which helps us to better understand the processes of plant stress acclimation and stress tolerance acquisition.

Materials and methods

Selection of proteins expressed in *Arabidopsis thaliana* salt-response proteins from Uniprot database

Uniprot (Release 2011_11, <http://www.uniprot.org>) was used to select *Arabidopsis thaliana* salt-response proteins. All *Arabidopsis thaliana* expressed proteins were extracted from Uniprot database to analyze GOTERM_BP_FAT. Subsequently, these proteins with key word "response to salt stress" in the description of GOTERM_BP_FAT were referred as salt-response proteins. The molecular function classification of salt-response proteins was performed according to GOTERM_MF_FAT.

Cytoscape (An Open Source Platform for Complex Network Analysis and Visualization) V2.8.2 analysis

Arabidopsis thaliana salt-response proteins were further analyzed by Cytoscape v2.8.2. Cytoscape is an open-source software package that is widely used to integrate and visualize diverse data sets in biology. By visualizing

information about known compound-target interactions in the context of a biological network of interest, one can rapidly identify novel avenues to perturb the system with compounds and potentially identify therapeutically relevant targets. Proteins with different cellular localizations and proteins involved in different stress were further analyzed by Cytoscape v2.8.2 (Srivastava et al., 2011).

Plant material, growth conditions and harvest

Arabidopsis thaliana (ecotype Col-0) seeds were germinated in a climate chamber under short-day conditions (8/16 h light/dark cycle, 22/20°C day/night, 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity) in plate with normal MS medium. After cultivation for 8 days under these conditions, *Arabidopsis thaliana* seedlings were respectively shifted to the cold-stress condition (10°C, day and night), transferred into drought-stress condition (MS medium supplemented with 200 mmol.L^{-1} mannitol), transferred into salt-stress condition (MS medium supplemented with 150 mmol.L^{-1} NaCl), and transferred into heavy metal-stress condition (MS medium supplemented with 10 $\mu\text{mol.L}^{-1}$ CdCl₂). Under the above four stress, *Arabidopsis thaliana* seedlings continued to grow for 4 days in a inversion way. After stress exposure, rosette leaves and roots were harvested, directly frozen in liquid nitrogen, and stored at -80°C until further processing. Three completely independent sets of harvests were included.

Enzyme Activity Assay

SOD activity was assayed by determination kit (Nanjing Jiancheng Bioengineering Institute NJBI). One unit of SOD activity was defined as the amount of enzyme required for 1mg tissue proteins in 1ml of a reaction mixture SOD inhibition rates to 50% as monitored at 550 nm. The activities of SOD were demonstrated with U $\cdot\text{mg}^{-1}$ protein. POD activity was determined by determination kit (Nanjing Jiancheng Bioengineering Institute NJBI). One unit of POD activity was defined as the amount of enzyme required for 1mg tissue proteins catalysed 1 μg substrate for 1 min as monitored at 420nm. The activities of POD were demonstrated with U $\cdot\text{mg}^{-1}$ protein.

Statistics analysis

Data were analyzed by t-test for significant differences between the two groups. The results were considered statistically significant when $p < 0.05$.

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