Integrated bioinformatics analysis reveals the response of Arabidopsis roots to salt stress through multiple gene expression omnibus datasets

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ABSTRACT

Plant growth and crop productivity has been adversely affected by salt stress around the world. Arabidopsis root is an important model to demonstrate how the glycophyte adapts to salt stress. To better understand the salt-responsive genes in Arabidopsis root, a rank aggregation method has been applied to disclose 836 dysregulated genes including 449 upregulated and 387 down-regulated genes out of more than 20,000 genes in NaCl-treated Arabidopsis root from the Gene Expression Omnibus (GEO) database. Then, the integrated approaches of Panther, DAVID, KEGG, Cytoscape and STRING have been used for bioinformatic analysis. Our results showed that these salt-responsive genes were involved in various gene ontology enrichments (transcription, oxidative stress, cell wall organization), differential pathways (MAPK signaling pathway, hormone signal transduction), and complicated interactions (cross-talking with wounding stress, abscisic acid stress, heat stress, water deprivation stress, cold stress). Furthermore, the activities of the oxidative stress related gene, peroxidase, were determined under salt stress, cold stress and water deprivation stress, which validated the bioinformatic outcome of cross-tolerance to different stresses. Our work will throw new lights on the response of Arabidopsis roots to salt stress, and provide potential salt-targets to improve plant growth and increase crop output.

Introduction

Soil salinity is a risk factor threatening plant growth and crop output (Guo et al. 2012a). Plants have developed many approaches including sodium exclusion and sodium ion accumulation tolerance to adapt to salt stress (Guo et al. 2012b). In order to demonstrate the mechanisms of salt-response, many researches have been performed with the model plant Arabidopsis (Guo et al. 2019). Two-dimensional electrophoresis has been used to discover the differential Arabidopsis root proteins between wild type and salt-tolerance mutant, which identified 19 differential proteins related to signal transduction, redox homeostasis, and cell wall metabolism (Guo et al. 2014b). Furthermore, phosphorylation variations of proteins from Arabidopsis root under salt stress also have been determined by Pro-Q Diamond stain. Nonsynchronous differences between protein abundance and protein phosphorylation indicated the complicated mechanisms of root salt-responsive proteins. These altered proteins were associated with reactive oxygen species scavenging and metabolisms of the cell wall (Guo et al. 2014a). Compared with proteomic strategy, transcriptomic researches could provide complementary information in plant salt-response.

For the better understanding on salt-response in Arabidopsis root, differentially expressed genes (DEGs) in response to salt stress were retrieved from the gene expression omnibus database (GEO). Among more than 20,000 Arabidopsis genes, 836 dysregulated genes were screened based on a rank aggregation method, and further analyzed by the bioinformatic tools of PANTHER, DAVID, KEGG, Cytoscape, and STRING. The prediction of bioinformatic analysis was confirmed by experiment. The altered genes will facilitate the salt stress tolerance researches and provide new targets for the better explanation of the salt adaption in the plant root.

Materials and methods

Retrieval of salt-responsive DEGs in Arabidopsis roots from GEO database

Microarray data (GSM184831, GSM184832, GSM184839, GSM184840, GSM184842, GSM184844, GSM184846, GSM184907, GSM184893, GSM184912, GSM184913, GSM184899, GSM184902, GSM184908, GSM184924, GSM184930, GSM218286, GSM1126230, GSM1126231, GSM1126232, GSM184894, GSM184901, GSM184903, GSM184904, GSM184905, GSM184906, GSM184907, GSM184908, GSM184909, GSM184910, GSM184911, GSM184912, GSM184913, GSM184914, GSM184915, GSM184916, GSM184917, GSM184918, GSM184919, GSM184920, GSM184921, GSM184922, GSM184923, GSM184924, GSM184925, GSM184926, GSM184929)

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Arabidopsis root; salt stress; cross-talking; bioinformatic analysis; experimental validation; gene expression omnibus; oxidative stress

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GSM1126236, GSM1126237, GSM1126238, GSM1824866, GSM1824867, GSM1824868, GSM1824869, GSM1824870, GSM1824871) related to salt-responsive gene expression profiling of Arabidopsis roots were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). Then, the data were calibrated by the online GEO tool of GEO2R (https://www.ncbi.nlm.nih.gov/geo2r/). The integration of DEGs from different datasets were performed by the robust rank aggregation (RRA) software package (Niu et al. 2019).

**Gene ontology classification**

The online bioinformatic tools of DAVID (Database for annotation, visualization and integrated discovery, Version 6.8) (https://david.ncifcrf.gov/) was applied for gene ontology enrichment analysis of the recruited proteins.
Pathway analysis

The pathways of salt-responsive *Arabidopsis* root genes were analyzed by the online program of KEGG (Kyoto Encyclopedia of Genes and Genomes, Release 94.0, 1 April 2020) (http://www.kegg.jp).

PPI network

The PPI interaction networks were constructed by the software of STRING (search tool for recurring instances of neighboring genes) database (Version 11.0, released 19 January 2019) (http://string-db.org/), and Cytoscape (an open source platform for complex network analysis and visualization, Version 3.7.2) (https://cytoscape.org/).

Growth conditions and harvest of *Arabidopsis* root

According to the previous publication (Guo et al. 2014b), the ecotype Col-0 *Arabidopsis thaliana* seeds were germinated on the normal medium plate with 22/20 °C day/night, 8/16 h light/dark cycle, 60 μmol·m⁻²·s⁻¹ light intensity. After 7 days,
the Arabidopsis seedlings were separately transferred into three stresses: cold-stress condition (8 °C), water-deprivation stress condition (200 mmol·L-1 mannitol), and salt stress condition (150 mmol·L-1 NaCl) for another 3 days growth. Then the Arabidopsis roots were harvested and stored at –80°C.

Enzyme activity assay

According to the previous method (Guo et al. 2019), peroxidase (POD) activity of Arabidopsis root was assayed using the kit (Nanjing Jiancheng Bioengineering Institute). Briefly, the POD activity was determined based on the catalyzing H2O2 of absorbance change at 420 nm. The plots were drawn with the software of Graphpad Prism 8 for windows (Version 8.0).

Results

Identification of salt-responsive DEGs in Arabidopsis root

In the present work, 16 salt-responsive experiments in Arabidopsis root were retrieved from the GEO database (Figure 1). In the experiment of salt stress-1 (GSM184831, GSM184832, GSM184839, GSM184840 data; (B) GSM184833, GSM184834, GSM184841, GSM184842 data; (C) GSM184835, GSM184836, GSM184843, GSM184844 data; (D) GSM184837, GSM184838, GSM184845, GSM184846 data; (E) GSM184839, GSM184849, GSM184890, GSM184891, GSM184907, GSM184908, GSM184909 data; (F) GSM184892, GSM184893, GSM184894, GSM184910, GSM184911, GSM184912; (G) GSM184895, GSM184896, GSM184897, GSM184913, GSM184914, GSM184915 data; (H) GSM184908, GSM184909, GSM184916, GSM184917, GSM184918 data; (I) GSM184901, GSM184902, GSM184903, GSM184919, GSM184920, GSM184921 data; (J) GSM184904, GSM184905, GSM184906, GSM184922, GSM184923, GSM184924 data; (K) GSM184925, GSM184926, GSM184927, GSM184929, GSM184930 data; (L) GSM218283, GSM218284, GSM218285, GSM218286 data; (M) GSM1126230, GSM1126231, GSM1126232, GSM1126234, GSM1126236, GSM1126237, GSM1126238 data; (N) GSM1126302, GSM1126303, GSM1126304, GSM1126307, GSM1126309, GSM1126310 data; (O) GSM1126315, GSM1126316, GSM1126317, GSM1126337, GSM1126338 data; (P) GSM1824866, GSM1824867, GSM1824868, GSM1824869, GSM1824870, GSM1824871 data. The value in the box is the logFC value, and “NA” represent the P-value > 0.05.
in *Arabidopsis* root (Figure 2(C)). In the experiment of salt stress-4 (GSM184837, GSM184838, GSM184845, GSM184846), 1073 down-regulated and 929 up-regulated DEGs were screened in *Arabidopsis* root (Figure 2(D)). In the experiment of salt stress-5 (GSM184889, GSM184890, GSM184891, GSM184907, GSM184908, GSM184909), 2251 down-regulated and 3558 up-regulated DEGs were screened in *Arabidopsis* root (Figure 2(E)). In the experiment of salt

![Figure 4](image)

**Figure 4. GO classification of differential salt-responding genes in *Arabidopsis* root.** (A) biological process classification of up-regulated salt-responsive genes, (B) biological process classification of down-regulated salt-responsive genes, (C) molecular functional classification of up-regulated salt-responsive genes, (D) molecular functional classification of down-regulated salt-responsive genes.

![Figure 5](image)

**Figure 5. KEGG analysis of differential salt-responding genes in *Arabidopsis* root.** (A) KEGG enrichment analysis of up-regulated genes (yellow colored) and down-regulated genes (blue colored) in *Arabidopsis* root. Circle size represents the number of genes, and circle color represents the P-value, (B) the representative up-regulated MAPK signaling pathway in *Arabidopsis* root, (C) the representative up-regulated hormone signal transduction pathway in *Arabidopsis* root.
stress-6 (GSM184892, GSM184893, GSM184894, GSM184910, GSM184911, GSM184912), 2506 down-regulated and 975 up-regulated DEGs were screened in Arabidopsis root (Figure 2(F)). In the experiment of salt stress-7 (GSM184895, GSM184896, GSM184897, GSM184913, GSM184914, GSM184915), 2052 down-regulated and 6078 up-regulated DEGs were screened in Arabidopsis root (Figure 2(G)). In the experiment of salt stress-8 (GSM184898, GSM184899, GSM184900, GSM184916, GSM184917, GSM184918), 815 down-regulated and 734 up-regulated DEGs were screened in Arabidopsis root (Figure 2(H)). In the experiment of salt stress-9 (GSM184901, GSM184902, GSM184903, GSM184919, GSM184920, GSM184921), 3467 down-regulated and 1946 up-regulated DEGs were screened in Arabidopsis root (Figure 2(I)). In the experiment of salt stress-10 (GSM184904, GSM184905, GSM184906, GSM184922, GSM184923, GSM184924), 2683 down-regulated and 1879 up-regulated DEGs were screened in Arabidopsis root (Figure 2(J)). In the experiment of salt stress-11 (GSM184925, GSM184926, GSM184929, GSM184930), 738 down-regulated and 941 up-regulated DEGs were screened in Arabidopsis root (Figure 2(K)). In the experiment of salt stress-12 (GSM218283, GSM218284, GSM218285, GSM218286), 876 down-regulated and 1212 up-regulated DEGs were screened in Arabidopsis root (Figure 2(L)). In the experiment of salt stress-13 (GSM1126230, GSM1126231, GSM1126232, GSM1126236, GSM1126237, GSM1126238), 633 down-regulated and 774 up-regulated DEGs were screened in Arabidopsis root (Figure 2(M)). In the experiment of salt stress-14 (GSM1126302, GSM1126303, GSM1126304, GSM1126308, GSM1126309, GSM1126310), 1409 down-regulated and 1026 up-regulated DEGs were screened in Arabidopsis root (Figure 2(N)). In the experiment of salt stress-15 (GSM1126335, GSM1126336, GSM1126337, GSM1126338), 2034 down-regulated and 2284 up-regulated DEGs were screened in Arabidopsis root (Figure 2(O)). In the experiment of salt stress-16 (GSM1824866, GSM1824867, GSM1824868, GSM1824869, GSM1824870, GSM1824871), 578 down-regulated and 595 up-regulated DEGs were screened in Arabidopsis root (Figure 2(P)). Further, the RRA method was applied to integrate the above DEGs. As a result, 449 up-regulated and 387 down-regulated genes were screened as the salt-responsive DEGs of Arabidopsis root (Figure 3, supplementary table 1).

Bioinformatics enrichment of salt-responsive DEGs in Arabidopsis root

Bioinformatics tools were used to analyze the identified 449 up-regulated and 387 down-regulated genes. Regarding
biological processes, the significantly enriched processes of up-regulated DEGs were related to regulation of transcription, protein dephosphorylation, response to oxidative stress (Figure 4(A)), whereas, the significantly enriched processes of down-regulated DEGs were associated with cell wall organization, root hair elongation, sterol metabolic process (Figure 4(B)). In terms of molecular function, the significantly enriched functions of up-regulated DEGs were related to transcription factor activity, glutathione transferase activity, kinase activity (Figure 4(C)), whereas, the significantly enriched functions of down-regulated DEGs were associated with phosphatase inhibitor activity, pectinesterase inhibitor activity, heme binding (Figure 4(D)). KEGG analysis indicated the up-regulated DEGs were involved in plant hormone signal transduction, metabolic pathways, MAPK signaling pathway, linoleic acid metabolism, glutathione metabolism, galactose metabolism, carotenoid biosynthesis, biosynthesis of secondary metabolites, amino sugar and nucleotide sugar metabolism, and the down-regulated DEGs mainly participated in pentose and glucuronate interconversions, and diterpenoid biosynthesis (Figure 5).

**PPI network and cross-talk of salt-responsive DEGs in Arabidopsis root**

Totally, 449 up-regulated salt-responsive DEGs were connected with 879 edges (PPI enrichment p-value: < 1.0e-16) by STRING (Figure 6). In addition, the complicated cross-talks of up-regulated salt-responsive genes with wounding stress, cold stress, abscisic acid stress, heat stress, and water deprivation stress in Arabidopsis root were shown with Cytoscape (Figure 7).

**POD activity determination in Arabidopsis root under salt, cold, and water deprivation stress**

Our results showed POD was not only an up-regulated salt-responsive gene, but also was involved in other stresses. Therefore, POD activity of Arabidopsis root were determined under three stresses (salt, cold, and water deprivation stress) to confirm the confidence of bioinformatics prediction. The results indicated that compared with the stress-free control, POD activity was all increased at different time points (12, 24, 36, 48, 60, 72 h) under salt, cold, and water deprivation stresses (Figure 8).

**Discussion**

Salt stress limits plant growth and decreases crop output on the earth. Many works have been done to improve the salt-tolerance of plants (Shah et al. 2018). Although several plants have been used as materials, Arabidopsis is the most ideal model to demonstrate the salt-response mechanisms (Zhang et al. 2018). The root of Arabidopsis was an organ dealing with salt stress directly. Therefore, many researches about salt adaption in the Arabidopsis root have been performed. For example, phosphorylation dynamics in Arabidopsis roots proteins have been detected, and 77 novel phosphorylated sites were found, which might be involved in lipid signaling under abiotic stress responses (Vialaret et al. 2014). Another experiment about the Arabidopsis root response to various long-term salt stress indicated that significant changes in both physiological and proteome level have occurred (Carrera et al. 2018).

Although some work has explored the molecular mechanisms of plant salt-tolerance, more researches are still needed...
to provide further information. In the present work, we used an integrated approach to disclose how the plant deals with salt at the transcription level. In comparison with the proteomic data, we considered the transcription data could give the complementary information to facilitate our better understanding on the plant salt-response.

Here, to overcome and minimize the heterogeneity of results caused by different experimental conditions, 16 salt-responsive experiments in Arabidopsis root were retrieved from GEO database. To integrate the DEGs results more efficiently, the RRA method has been used to screen the DEGs list. As the result, 836 dysregulated genes including 449 up-regulated and 387 down-regulated genes were defined as the salt-responsive DEGs in the Arabidopsis root.

Based on the analysis of bioinformatics tools, both up-regulated DEGs and down-regulated DEGs were involved in different biological process, molecular function and KEGG pathway. For example, in the up-regulated DEGs, response to oxidative stress was a significantly enriched biological process, and glutathione metabolism was a significantly enriched KEGG pathway. Usually, salt stress will attenuate the electron transport chain and produce excessive ROS, such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals (Miller et al. 2010). ROS scavenging-related enzymes including glutathione S-transferase family (Chan and Lam 2014) and POD (Islam et al. 2015) were activated to maintain the balance of redox homeostasis in plant cells. In the present work, POD, glutathione S-transferase tau 2 (GSTU2), glutathione S-transferase tau 3 (GSTU3), glutathione S-transferase tau 4 (GSTU4), glutathione S-transferase tau 5 (GSTU5), glutathione S-transferase tau 6 (GSTU6), glutathione S-transferase tau 9 (GSTU9), Glutathione S-transferase family protein (ERD9) were all up-regulated in response to the salt stress.

Based on the annotation of PPI network, some DEGs were involved in several stresses, which was named stress cross-talk (Tuteja 2007). A previous work has reported that some salt-inducible genes were related to the cold stress (Seki et al. 2002). In the present work, the cross-talk of different stresses were more complicated than the previous finding. The salt-responsive DEGs were associated in wounding stress, cold stress, abscisic acid stress, heat stress, and water deprivation stress, which dramatically improved our understanding on salt-tolerance mechanisms in Arabidopsis root. To validate the cross-talk among different stresses, the enzyme activity of POD in root was assayed under salt stress, cold stress and water deprivation stress, individually. And the results agreed with the stress cross-talk prediction from the integrated bioinformatics analysis.

Conclusion
This work gave new light on the understanding of salt responses in Arabidopsis root. Those pathways, PPI networks, stress cross-talking would provide more important information to discover the potential salt-responsive biomarkers. In the future, more work is still needed to explore the functions of DEGs to better improve plant growth and crop output.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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