Comparative Transcriptomics Analysis for Rice Seedlings of Contrast Salt-tolerant Cultivars

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Abstract
Salt stress is a severe problem that affects rice cultivation and production. To understand the rice salt-tolerance mechanism, we used microarray to compare the gene expression profiling of seedlings between two salt-tolerant rice cultivars. The phenotype and physiological analysis of TNG67 (Japonica) and TCN1 (Indica) seedlings under salt stress showed that TNG67 were more salt-tolerant than TCN1. The global gene expression profile analysis revealed that ABA, ethylene, cytokinin and polyamine played important roles in the salt stress tolerance of rice. In addition, we found that the main transcription factors (TFs) expressed differentially in shoots under salt stress were BZIP and ERF genes instead of NAC genes in roots. In particular, the WRKY genes expressed dominantly in roots under salt stress but not in shoots. These results indicated that both shoots and roots may mediate salt responsiveness and tolerance through different mechanisms and shoot-root communication was essential for salt tolerance of rice seedlings. Besides, TFs that expressed preferentially in TNG67 under salt stress but not in TCN1 at early stage of salt stress may represent as candidate key genes for salt tolerance and worthy for further study.

I. Introduction
Salt stress is one of the crucial problems for plants, especially in arid irrigated fields because salt affects crop growth and production. Salt tolerance is an important agronomical trait for stable rice yield. The rice seedling stage is vulnerable to salt stress. Thus, there is an increase demand on dissecting the molecular mechanism of salt tolerance in rice seedling. To investigate chilling tolerance mechanisms of rice, here we reported a comprehensive transcriptomics analysis of two rice genotypes (salt-tolerant TNG67 and salt-sensitive TCN1) with contrast salt responses.

II. Material and Methods
RNA samples were extracted from rice seedlings in shoot and root separately at three-leaf stage under salt (250 mM) treatment for 3, 24 hours and recovery for 24 hours. The microarray Rice OneArray™ v1 from Phalanx Biotech Group was designed for Japonica Group and Indica Group of Oryza sativa; focusing on environmental stress resistance gene and well-annotated gene. To obtain appropriate data for probe designing, the MSU v6.1 DNA sequences, BGI Indica (93-11) coding sequences and BGI Japonica (Syngenta) coding sequences were used as the probe target. The data were analyzed with the Rosetta Resolver® gene expression data analysis system and data were considered as Differential Expression Gene (DEG) while the log of fold change of treatment/control was used to create the heatmap. Gray color indicate there is no signal in the microarray chip of this gene probe.

III. Results

A. Shoot

- Figure 1. The injury test of TCN1 and TNG67 rice seedlings on the measurement of (A) Fv/Fm and (B) leakage conductivity after salt treatment (250 mM) for 24 hrs and then recovery at 3 days.

- Figure 2. Hierarchical clustering analysis of expression profile of differential expression genes (DEG). Based fold changes of treatment/control was used to create the heatmap (A) in shoot and root (B) of TNG67 and TCN1.

- Figure 4. GO annotation of differentially expressed genes for BP-Biological process after salt treatment and recovery. Bars show numbers of genes in each GO slim category. GO enrichment was analyzed with agriGO (http://bioinfo.cau.edu.cn/agriGO) and GO terms were marked with Χ which considered to be not significantly different in the cluster.

- Figure 6. Analysis for DEGs of TFs under salt stress for 3 hrs. (A) Venn diagram of DEGs of TF genes (lower area below the blue line and the amount written in dark grey color) in TNG67 and TCN1 under salt stress conditions in different tissues. (B) The number of major TFs group expressed differentially in shoots. (C) The number of major TFs group expressed differentially in root.

1. Conclusion
We found the DEGs under salt treatment varied dramatically not only within two cultivars, but also for different tissues of shoot and root. There are more TFs expressed differentially in root of TNG67 rice variety. This result may indicate that the root play an important role for TNG67 rice variety to cope with salt stress. Most of these TFs had been shown to be regulated by hormones on RiceXPro (http://ricexpro.dna.affrc.go.jp/) that also implied the phytohormones could participate in salt-tolerant mechanism. This is corresponding to the results of our microarray analysis for the expression of hormone-related genes. Further analysis showed that these TFs and hormone-related genes can provide us some clues for further study to elucidate the salt stress tolerance mechanism in TNG67.