Elucidation of the Salt Tolerance Mechanism of Rice by Profiling the Transcriptome in Sea Rice 86 Root

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Abstract

Background and aim

In order to explore the molecular mechanism of salt tolerance of rice (*Oryza sativa L*.

Methods

the transcriptome of 0.9% NaCl treated seawater rice roots was sequenced with Sea Rice 86 with strong salt tolerance.

Results

The results showed that 1055 differential genes were obtained by transcriptome sequencing, of which 691 differential genes were up-regulated and 364 differential genes were down regulated. Through go analysis of differentially expressed genes, it was found that salt stress caused differential expression of genes related to metabolic process, cell process, protein binding, catalytic activity, antioxidant activity and cell separation of Sea Rice 86, which may be Sea Rice 86 played an important role in root resistance to salt stress; The differentially expressed genes were annotated with KEGG function, The differentially expressed genes in plant hormone signal transduction, galactose metabolism, glutathione metabolism, plant pathogen interaction and glycolysis / gluconeogenesis were compared after 3 and 0 days of salt treatment (glycolysis / gluconeogenesis), phenylpropanoid biosynthesis, etc. The results of differentially expressed genes were consistent with the transcriptome data verified by qRT-PCR. There were significant differences in ion content, proline and peroxide metabolism in seawater rice roots under salt stress.The molecular mechanism of salt stress in the roots of Sea Rice 86 was deeply discussed, which provided a basis for further application in genetic engineering and molecular breeding.

Introduction

Salt stress is one of the major abiotic stresses affecting the plant growth, development and yield(Wu. 2020; Wang,2019). According to the incomplete statistics of UNESCO and FAO, the area of saline alkali land in the world is 954 million hm^2, which seriously affects the sustainable development of irrigation agriculture in the world(Li,2005). The existing saline alkali land area in China is as high as 9.9 × 106 hm2, of which those with agricultural development potential account for more than 10% of the total cultivated land area in China(Gao,2021). Rice is an important food crop in China(Goswami, 2017). Rice production is closely related to China's national economy. Improving the utilization rate of saline alkali land in China is an important measure to improve rice yield(Song,2018).

Sea Rice 86 is an important saline alkali tolerant rice germplasm resource, which was first discovered and named by Chen Risheng at the seaside of Zhanjiang in 1986(Yang,2018). In 2016, Yang Jun and others used indel molecular index method to analyze the indica japonica character of Sea Rice 86(yang, 2016). The results showed that Sea Rice 86 was an indica rice, and it was preliminarily determined that it was

not a wild rice(Yang,2018). The study of salt tolerance mechanism of Sea Rice 86 is of great significance for rice molecular breeding.

Transcriptome sequencing (RNA-Seq) technology is to sequence cell transcripts, count each sequence measured, and obtain the expression of each specific transcript. It can more accurately evaluate cell phenotype, deepen the understanding of cell metabolism, help to improve target genes, and become an important means of molecular breeding(Zhang,2021). In recent years, the salt tolerance mechanism of Sea Rice 86 has been studied, but it is not well understood, and most of the studies focus on the leaves, but less on the roots. In this study, transcriptome sequencing (RNA-Seq) technology was used to sequence the transcriptome of Sea Rice 86 roots before and after salt stress, in order to obtain and analyze the transcriptome data of Sea Rice 86 roots, provide a theoretical basis for the subsequent research on the molecular level such as exploring the salt tolerance functional genes of rice, elaborating the salt tolerance mechanism and regulation mechanism, and provide an improved idea for the molecular breeding of salt tolerant rice.

Materials And Methods

Plant Materials and salt stress resistance assay

Sea Rice 86, which grew to three leaf one heart stage, was used as experimental material. The seedlings were treated with normal International Rice Institute nutrient solution and International Rice Institute nutrient solution containing 0.9% NaCl (aq). Taking the material (CK) treated with normal nutrient solution as the control group and the material treated with 0.9% NaCl (aq) nutrient solution as the treatment group, every three rice plants are set as one biological repeat, and three biological repeats are set. The roots (CK1, CK2, CK3, T1, T2 and T3) are taken respectively. After rapid freezing with liquid nitrogen, they are placed in the refrigerator at − 80 ℃.

RNA extraction, reverse transcription and quantitative RT-PCR

Roots of sea rice 86 control and treated groups were sampled separately, and RNA was extracted and reverse transcribed for quantitative fluorescence validation.The qRT-PCR was performed with Actin as the internal reference, and the relative expression amount was calculated with $2^{-\Delta\Delta CT}$.

RNA Library Construction

Eukaryotic mRNA was enriched with magnetic beads with oligo (DT), and then the mRNA was randomly interrupted by adding fragmentation buffer (random hexamers) synthesized the first cDNA strand, then added buffer, dNTPs, RNase H and DNA polymerase I to synthesize the second cDNA strand, and purified the cDNA with ampure XP beads. The purified double stranded cDNA was subjected to terminal repair, added a tail and connected to the sequencing connector, then selected the fragment size with ampure XP beads, and finally enriched to obtain the cDNA library by PCR.

Sequencing and data assembly

After passing the library inspection, high-throughput sequencing was performed with hiseq2500, and the sequencing reading length was PE100. Raw data is obtained for data filtering, the joint sequence and low-quality reads are removed, and high-quality clean data is obtained. The sequence is assembled according to Trinity (http: / / trinityrnaseq. SourceForge. Net /).

UniGene function notes

Use the blast comparison tool to compare the sample UniGene with the public database, make functional annotation through gene similarity, compare the sample gene sequence with NR, Swiss prot, KEGG, COG and go databases respectively, and set E-value ≤ 10 − 5 to obtain annotation information.

Analysis of differentially expressed genes

Using ebseq (HTTPS: / / www.biostat. WISC. Edu / ~ kendzior / ebseq /) for differential expression analysis, FDR was used as the index for differential expression gene screening to reduce false positives. In the screening process, FDR < 0.01 and differential multiple FC ≥ 2 were used as the screening criteria, FC represents the ratio of expression between the two samples. Go and cog were performed for differentially expressed genes to And KEGG annotation, and the differentially expressed genes were further analyzed by David annotation tool.

Result

Sequencing results and sequence assembly

A total of 41.11 Gb clean data was obtained, the clean data of each sample reached 6.32 Gb, and the percentage of Q30 base was 93.65% or more.

Analysis of differentially expressed genes

A total of 1055 unigenes were differentially expressed in the two groups of samples, of which 691 were up-regulated and 364 were down regulated, which may be due to plant growth differences caused by salt stress. The detailed annotation results of these differentially expressed genes are shown in Table 2.

GO annotation analysis of differentially expressed genes

Through go classification of differentially expressed genes (Fig. 1), 1764 unigenes are annotated into three categories: biological process, cellular component and molecular function. In biological process, metabolic process□cellular process□ single- organism process□ response to stimulus□ biological regulation and localization are the species with the most enrichment of differentially expressed genes after salt stress treatment; among the major categories of molecular functions, binding□catalytic activity□ transporter activity□nuclear acid binding transcription factor activity□electron carrier activity□antioxidant activity and signal sensor activity is the kind with the most enrichment and annotation of differentially

expressed genes; among the cell components, cell part cell organelle membrane membrane part and organelle part. Through go analysis of differentially expressed genes, it is found that salt stress causes the differential expression of genes related to metabolic process cellular process binding catalytic activity antioxidant activity and cell part of Sea Rice 86, which may play an important role in the root resistance to salt stress.

COG analysis of differentially expressed genesing

The differentially expressed genes were further classified by COG (Fig. 2), and 426 differentially expressed genes were compared to the COG database. Among them, the more enriched differentially expressed genes were Signal transduction mechanisms (62)Carbohydrate transport and metabolism(59)Posttranslational modification, protein tuner, chaperones(42)Defense mechanisms(41), Secondary metabolites biosynthesis, transport and catabolism(37) and General function prediction only(37).From this information, it can be found that salt stress caused the differential expression of growth, development and metabolism related genes of Sea Rice 86. At the same time, salt stress also caused the differential expression of genes related to Signal transduction mechanism, Posttranslational modification and transport, molecular chaperone and Secondary metabolites biosynthesis, transport and catabolism of Sea Rice 86. This information has important guiding significance for us to deeply understand the salt stress response mechanism of Sea Rice 86.

KEGG annotation of differentially expressed genes

In order to obtain the regulation information of salt stress of Sea Rice 86, all unigenes assembled in the two databases were annotated into KEGG database, and a total of 260 unigenes obtained annotation information, Among them, the pathways with the most enrichment of differentially expressed genes are Plant hormone signal transduction, Galactose metabolism, Glutathione metabolism, Plant-pathogen interaction, Glycolysis / Gluconeogenesis and Phenylpropane biosynthesis(Fig. 3).From this information, it can be concluded that salt stress of Sea Rice can cause changes in Plant hormone signal transduction, Glutathione metabolism and Pathogen response related pathway genes in seedling leaves, which is very helpful for us to understand the gene expression regulation of Sea Rice 86 under different stress conditions.

Analysis of transcription factors under Cadmium Stress

There have been many reports on the changes of different transcription factors caused by salt stress in many plants, including Arabidopsis thaliana. By comparing with Arabidopsis database, we obtained a total of 1968 annotated transcription factors (Fig. 4). By analyzing these transcription factors, we found that NAC, bHLH, AP_2 /ERF-ERF, C_2H_2, MYB and WRKY families have more annotation information.

Validation of the qRT-PCR expression

According to the functional comparison of differentially expressed genes under salt stress, five genes involved in salt treatment for 3 d and 0 d were selected and tested by quantitative real-time fluorescence using Actin as the internal reference.The comparative expression of the five genes in salt treatment with 3 d and 0d showed that all the six genes selected from 86 salt treatment with 3 d and 0d showed downregulated expression, and the results were consistent with the transcriptome data, indicating the true and reliable sequencing results(Fig. 5).

Discussion

The new generation sequencing technology has been applied in many plants, including millet, cotton and so on(Qi, 2011). In our study, Illumina sequencing technology was used to sequence the treatment group and the control group. The sequencing results of the control group and salt stress were compared. 1055 differentially expressed genes were found in the two samples, including 691 up-regulated genes and 364 down-regulated genes. The related expressed genes and metabolic pathways in 86 roots of Sea Rice were analyzed by go, COG and KEGG annotation. Compared with 9311 reference genome, it was found that salt stress caused the changes of ion transport related genes in Sea Rice 86 roots, affected the photosynthesis of Sea Rice 86, and led to the changes of Na and K ion homeostasis in roots. Through the analysis of differentially expressed transcription factors in Sea Rice 86 roots under treatment group and salt stress, it was found that NAC and bZIP transcription factors played an important role in Sea Rice 86 salt stress, and salt stress affected the changes of other stress-related genes of Sea Rice 86 to a certain extent.

Declarations

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“The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.”

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Tables

Table 1 Annotation of differential expression genes

□□□	COG	GO	KEGG	KOG	NR	Pfam	Swiss-Prot	eggNOG
□□□	426	904	260	484	1041	844	786	894

Figures

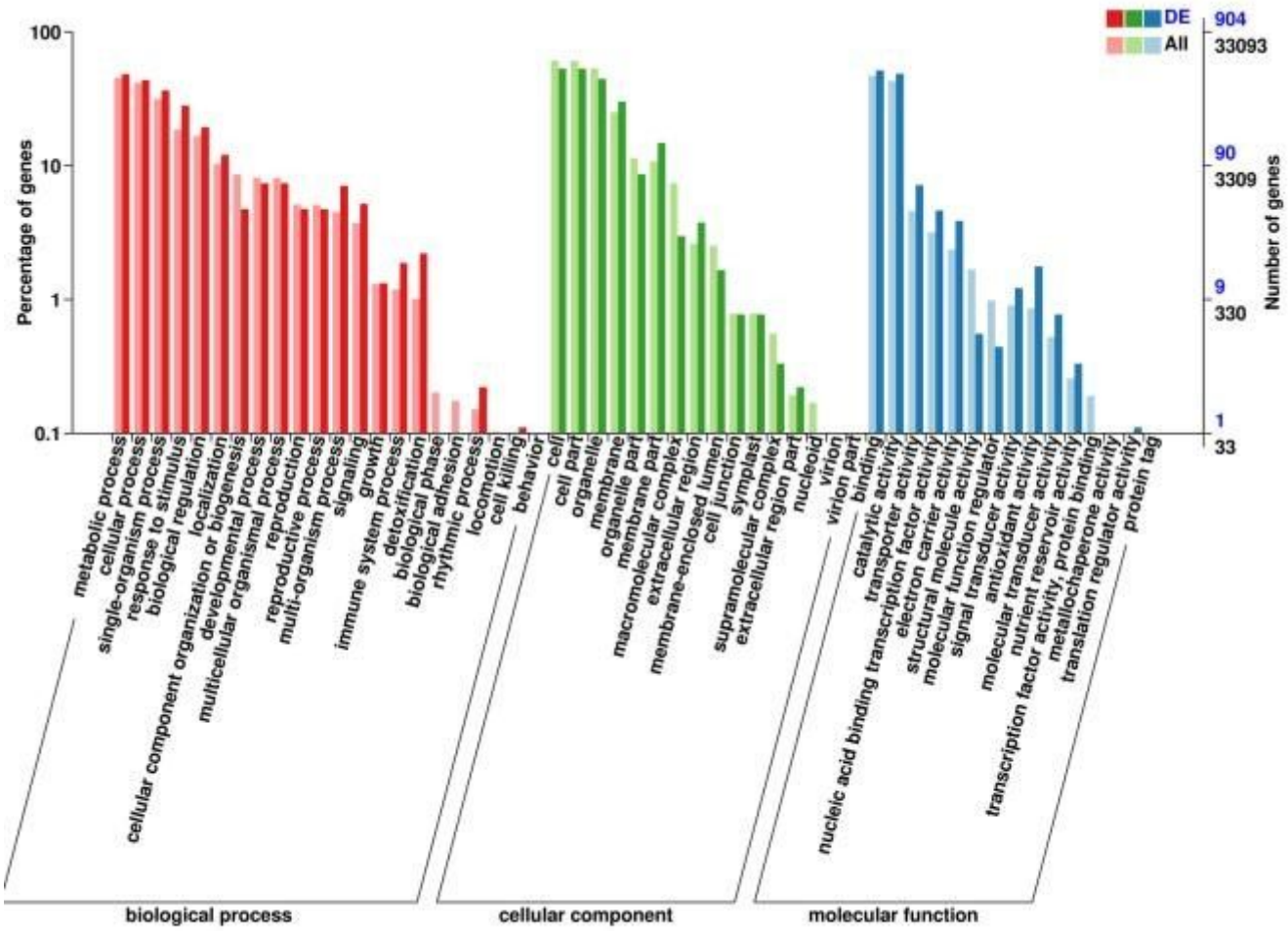

Figure 1

GO annotation of differential expression genes

X-axis indicaes number of Unigenes□y-axis indicates different GO terms

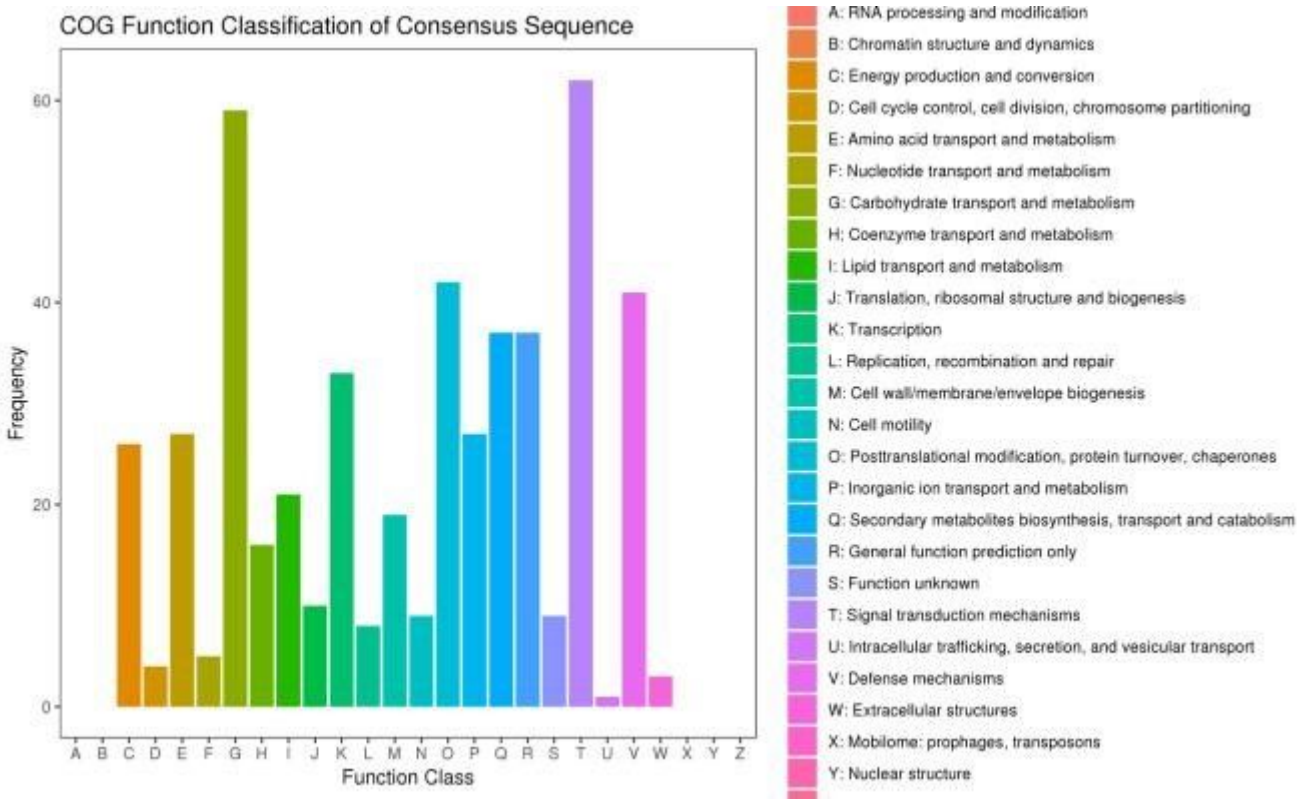

Figure 2

COG annotation of differential expression genes

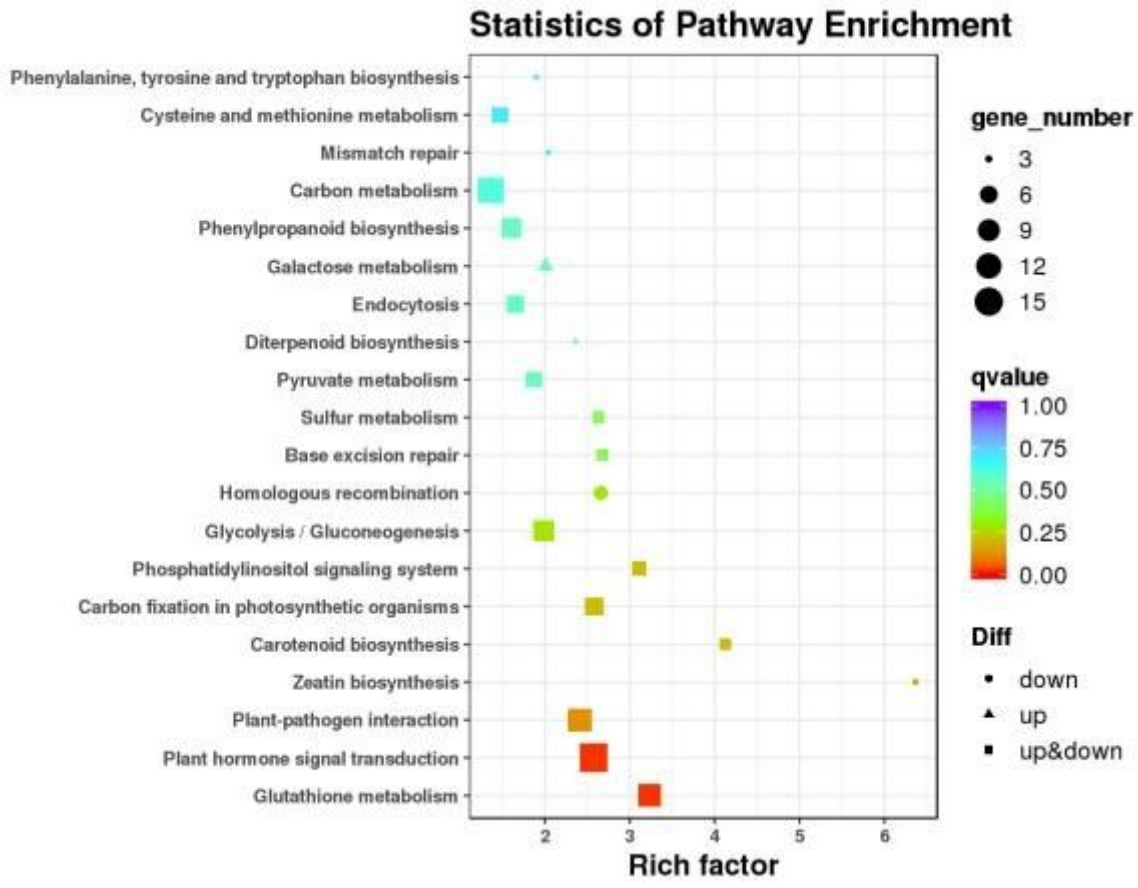

Figure 3

Sscatter diagram of enrichment of differentially expressed gene KEGG pathway

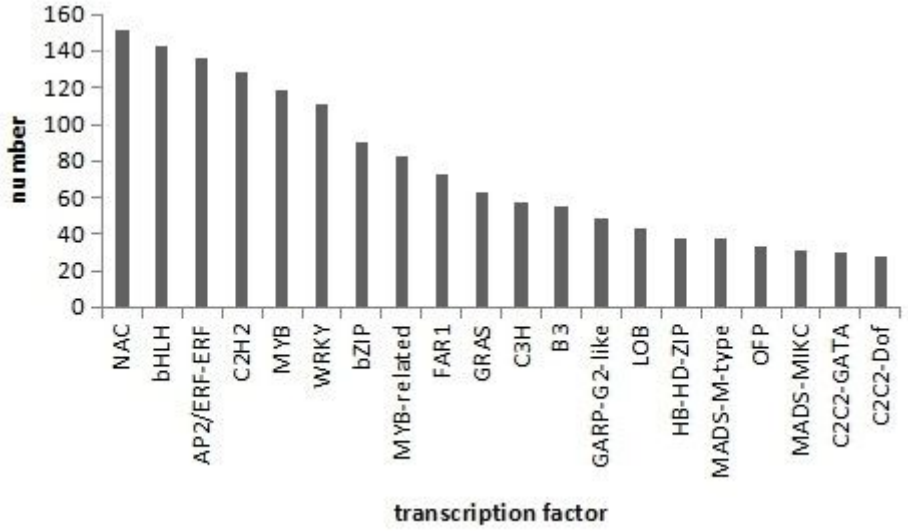

Figure 4

Sscatter diagram of enrichment of differentially expressed gene KEGG

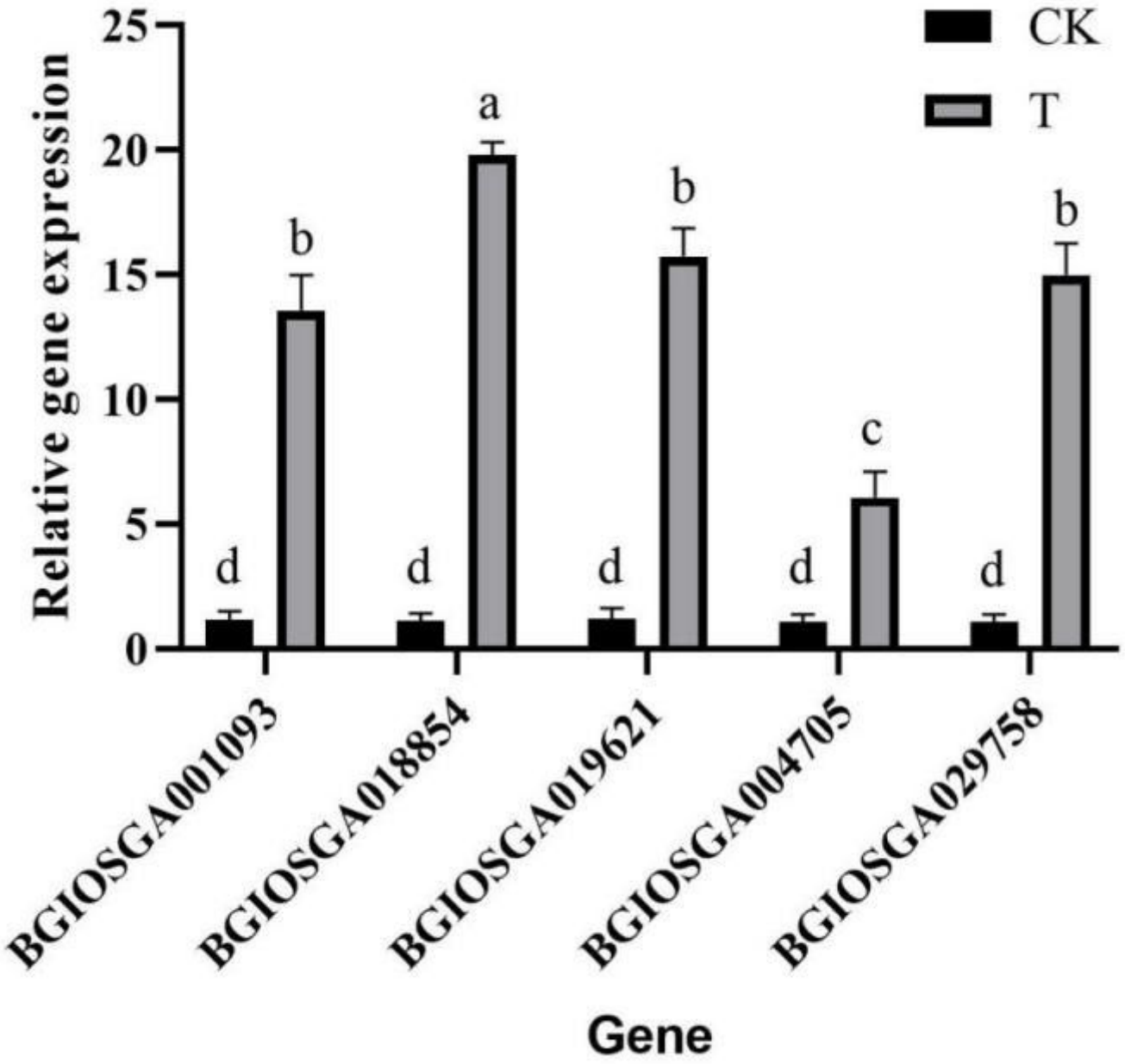

Figure 5

Relative expression of five differential genes