RNA-seq reveals that multiple plant hormones are regulated by Bacillus cereus G2 in Glycyrrhiza uralensis subjected to salt stress

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ABSTRACT

Bacillus cereus G2 can alleviate the adverse effect caused by salt in Glycyrrhiza uralensis Fisch. Transcriptome in the salt (S) treatment and control (CK) groups with or without G2 (CK + B or S + B) were sequenced to identify G2 regulate genes and response pathways under salt stress in G. uralensis. A total of 3608 differentially expressed genes (DEGs) were identified, of which 1589, 623, 469, and 927 DEGs were identified in S vs CK, CK + B vs CK, S + B vs S and S + B vs CK + B comparisons, respectively. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that G2 improved salt-tolerance mainly by regulating phytohormone in G. uralensis. Specifically, G2 up-regulated the DEGs related to auxin (IAA) signal transduction, abscisic acid (ABA) biosynthesis, signal transduction, and catabolism, and jasmonic acid (JA) biosynthesis and signal transduction under salt stress. Overall, multiple phytohormones were involved in the interaction of salt stress and G2 in G. uralensis.

Introduction

Glycyrrhiza uralensis Fisch, as a kind of Chinese herbal medicine and natural sweetener widely used in modern society, has been facing the threat of salt stress. Salinity affects about 7% of the global land and can ultimately inhibit crop production by limiting the amount of land under cultivation, which involves changes in various physiiological and metabolic processes, depending on the severity and duration of stress (Gupta and Huang, 2014; Long et al. 2015).

The regulation of plant hormones is related to plant salt tolerance (Ryu and Cho 2015). Some studies have confirmed that auxin (IAA) is involved in response to salt stress in plants (Iqbal et al. 2014; Ryu and Cho 2015) and a link between IAA signaling and salt stress has been established (Jung and Park 2011). Jasmonic acid (JA) is also involved in the salt response in wheat and that the a-linolenic acid metabolism pathway played a regulatory role in this response (Zhao et al. 2014). Some studies have indicated that abscisic acid (ABA) plays an important role as a signal molecule in the response of both the ice plant and Arabidopsis to salt stress (Thomas and Bohnert 1993; Tsukagoshi et al. 2015). EIN3 in ethylene (ET) signal transduction under salt stress can increase the transcription level of salt stress related-genes by activating the expression of ESI1, thus enhancing the salt tolerance of plants (Zhang et al. 2011). These results indicate that the hormone-mediated regulatory network is a key molecular mechanism of salt tolerance in various plants.

Endophytes are defined as microorganisms that live within plant tissues in the whole or part of their life cycle without causing any visible symptoms (Khan et al. 2017). Aspergillus flavus CHS1 significantly increased plant growth and alleviated salt stress by down-regulating the genes related to ABA and JA synthesis in Glycine max. L (Lubna et al. 2018). Yarrowia lipolytica FH1 has significantly improved the growth of maize under salt stress by regulating endogenous IAA and ABA (Gul Jan et al. 2019). Penicillium janthinellum LK5 helped the Sitiens plants to synthesis significantly higher ABA and reduced the level of JA to respond to salt stress (Khan et al. 2013). Pseudomonas spp. improved nutrient uptake and plant growth under moderate salt-affected conditions by reducing stress-related ET levels in tomato plants (Win et al. 2018). Penicillium produced bioactive gibberellins (GAs) to help plants overcome salt stress in many plants (Leitão and Enguita 2016). Bacillus amyloliquifaciens RWL-1 increased Oryza sativa salt tolerance by producing ABA (Shahzad et al. 2017). Aspergillus fumigatus sp. LH02 improved salt tolerance of Waito-C rice seedlings by decreasing ABA content and increasing the contents of salicylic acid (SA) and JA (Khan et al. 2011). Therefore, endophytes can improve plant salt tolerance and promote plant growth by influencing plant hormones.

Bacillus, as a Gram-positive model bacterium, is widely distributed in the leaves, roots, and stems of plants (Li et al. 2020). In our previous studies, Bacillus cereus G2 improved the growth of G. uralensis under salt stress, but the mechanism...
remains unknown. In this study, transcriptomes in the salt treatment and control groups with or without G2 were sequenced to identify G2 regulate genes and response pathways under salt stress in *G. uralensis*. The resulting data might help to better understand the underlying molecular mechanisms of G2 improve salt tolerance of *G. uralensis*.

**Materials and methods**

**Plant material**

*G. uralensis* seeds were collected from wild *G. uralensis* plants in Urad front flag, Inner Mongolia, China, in September, 2019. Healthy seeds were selected and stored in a kraft paper bag at 4°C until use.

**Bacterial culture suspension**

*Bacillus cereus* MT803148 (G2) was isolated from *G. uralensis* roots and identified by Sangon Biotech (Shanghai) Co., Ltd. G2 was cultured at 30°C for 2 days in Nutrient Agar (NA) medium, and then inoculated in the sterilized NA liquid medium. The bacteria culture suspension was incubated in a shaking incubator of 180 rpm at 28°C for 2 days until bacteria density reached 10^8 cfu mL^-1.  

**Salinity and B. cereus (G2) treatments**

Seeds of *G. uralensis* were steeped with concentrated sulfuric acid (H_2SO_4) for 1.5 h, then surface sterilized with 0.1% H_2O_2 for 10 min, rinsed 3 times in distilled water and soaked in distilled water for 9 h at room temperature. The experiment was arranged in a randomized experimental design and included: (i) Control group (CK), plants were watered with 100 mL of tap water; (ii) Control combined with G2 group (CK+B), plants were watered with 100 mL of tap water containing centrifuged bacteria culture suspension (10^8 cfu mL^-1 G2); (iii) Salt stress group (S), plants were watered with 100 mL of tap water containing 75 mM NaCl; (iv) Salt stress combined with G2 group (S+B), plants were watered with 100 mL of tap water containing 75 mM NaCl and 10^8 cfu mL^-1 G2. After the third true leaf of the seedling appeared, bacterial treatment started, and indicators were measured after 10 days.

**RNA extraction, cDNA library construction and RNA-Seq**

Transcriptome sequencing was carried out by the Beijing Baimeike Company. The experimental process follows the method provided by Oxford Nanopore Technologies (ONT), which mainly includes the following steps: i. Extract RNA and test its purity, concentration and integrity. ii. Library construction: Primer annealing, reverse transcription into cDNA, plus switch Oligo; Synthesis of complementary chains; DNA damage repair and terminal repair, magnetic bead purification. iii. Adding the sequencing connector for on-machine sequencing.

**Transcriptome data assembly**

Filter the low-quality (length less than 500 bp, Qscore less than 7) sequence and ribosomal RNA sequence from the original landing sequence, and obtain the full-length sequence according to the presence of primers at both ends of the sequence. Polish the full-length sequence obtained in the previous step to obtain the consistent sequence. Contig comparisons with reference genomes or constructed contig sequences were performed to remove redundancy.

**Transcription quantification**

Transcriptome sequencing can be simulated as a random sampling process. In order to make the number of fragments truly reflect the expression level of transcripts, it is necessary to normalize the number of mapped reads in the sample. CPM (counts per million) (Zhou et al. 2014) was used as an indicator to measure the expression level of transcripts or genes. The calculation formula of CPM was as follows (reads mapped to transcript means the number of reads compared to a transcript; total reads aligned in the sample represents the total number of fragments compared to the reference transcriptome):

$$CPM = \frac{\text{reads mapped to transcript}}{\text{total reads aligned in sample}} \times 1,000,000$$

**Criteria for differentially expressed genes (DEGs)**

The criterion for screening were a $|\log_2 \text{fold change}| > 1.5$ and a statistically significant q value < 0.05.

**Quantitative real-time PCR (qRT-PCR)**

qRT-PCR was performed on the same RNA pools, which were previously used in RNASeq for 12 randomly selected DEGs and 5 plant hormone-related DEGs in *G. uralensis*, respectively, to prove the reliability of the RNA-Seq data. Gene-specific primers were designed based on the sequencing data using the Primer-BLAST tool. The primer sequences used for qRT-PCR analysis are listed in Table S3 and Table S4. First-strand cDNAs were synthesized from 2 μg of DNase-treated total RNA using RevertAid RT Reverse Transcription Kit (ServiceBio, China). qPCR was performed by Wuhan servicebio technology CO., LTD using 2×SYBR Green qPCR Master Mix (High ROX). The reaction mixture (15 μL) contained 2×SYBR Green qPCR Master Mix (High ROX) 7.5 μL, 1.5 μL of primer, 2.0 μL of template cDNA, and 4.0 μL of ddH_2O. Amplifications were performed under the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Relative expression for each sample was calculated using the 2^−ΔΔ Ct methods with normalization to the internal control genes.

**Results**

**Transcriptome data analysis for the effects of G2 upon G. uralensis under salt stress**

**Gene annotation**

In this study, we carried out a comprehensive identification of transcriptional responses of *G. uralensis* to four different treatments by RNA-Seq (see the experimental design in the Methods section). To achieve comprehensive gene functional annotation, all assembled unigenes were blasted against public databases, including RefSeq non-redundant
proteins (NR), Protein family (Pfam), Clusters of Orthologous Groups of proteins (KOG/COG), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO) and evolutionary genealogy of genes: Non-supervised Orthologous Groups (eggNOG) (Table S1).

**Identification of DEGs**

A total of 35,831 genes in all samples of *G. uralensis* were identified and quantified by transcriptions, among which 3608 DEGs were identified. As shown in Table S2, compared with CK, there were 1589 DEGs in S treatment (S vs CK comparison), including 641 up-regulated and 948 down-regulated genes, and there were 623 DEGs in CK + B treatment (CK + B vs CK comparison), including 215 up-regulated and 408 down-regulated genes; Compared with S, there were 469 DEGs in S + B treatment (S + B vs S comparison), including 257 up-regulated and 212 down-regulated genes; Compared with CK + B, there were 927 DEGs in S + B treatment (S + B vs CK comparison), including 487 up-regulated and 440 down-regulated genes.

Validation of expression levels for 12 randomly selected DEG candidates was carried out by quantitative real-time PCR (qRT-PCR) (Table S3). The results showed high congruence between RNA-Seq and qRT-PCR results (coefficient of determination $R^2 = 0.9088$) indicating the reliability of RNA-Seq quantification of gene expression (Fig. S1).

**Functional classification of DEGs**

There were 118 core DEGs that appeared in both S vs CK and S + B vs S comparisons (Figure 1(A)). Among them, 38 DEGs were up-regulated and 80 DEGs were down-regulated in S vs CK comparison, while 73 DEGs were up-regulated and 45 DEGs were down-regulated in S + B vs S comparison (Figure 1(B)). The annotated number of these core DEGs varied in different public databases, with 36 DEGs annotated in COG database, 44 DEGs annotated in KOG database and 22 DEGs annotated in KEGG database (Figure 2).

Specifically, most core DEGs enriched in signal transduction mechanisms (8 DEGs), carbohydrate transport and metabolism (7 DEGs) and cell wall/membrane/envelope biogenesis (3 DEGs) in COG database; Most core DEGs enriched in secondary metabolites biosynthesis, transport and catabolism (6 DEGs), general function prediction only (6 DEGs), transcription (4 DEGs), posttranslational modification, protein turnover, chaperones (4 DEGs) and energy production and conversion (4) in KOG database; Most core DEGs enriched in starch and sucrose metabolism (2), plant-pathogen interaction (2 DEGs), amino sugar and nucleotide sugar metabolism (2 DEGs), fructose and mannose metabolism (2 DEGs) and plant hormone signal transduction (2 DEGs) in KEGG database.

Through GO analysis, GO term falls into three categories: biological process, molecular function and cellular component (Figure 3). Specifically, single-organism process, metabolic process, cellular process are the three most enriched GO terms in the biological process ontology of S vs CK and S + B vs S comparisons; Catalytic activity and binding are the two most enriched GO terms in the molecular function ontology of S vs CK and S + B vs S comparisons; Cell, membrane and cell part are the three most enriched GO terms in the cellular component ontology of S vs CK and S + B vs S comparisons. Since biological process and molecular function are closely related to plants’ internal mechanisms, GO analysis mainly focuses on these two parts. Through the venn diagram of five GO secondary annotations (single-organism process, metabolic processes, cellular process, catalytic activity, binding) with most DEGs in biological process and molecular function under S vs CK and S + B vs S comparisons, we found that some core DEGs were regulated both in S vs CK and S + B vs S comparisons (Figure 4(A)).

Through the KEGG analysis of the core DEGs in the above five secondary annotations under S vs CK and S + B vs S comparisons (Figure 4(B)), we found that these DEGs were dispersed but mainly concentrated in carbohydrate metabolism and other pathways.

In the first 20 KEGG enriched pathways, the DEGs in S vs CK comparison are mainly enriched in plant hormone signal transduction (25 DEGs), photosynthesis (21 DEGs), plant-pathogen interaction (20 DEGs), ribosome (19 DEGs), starch and sucrose metabolism (18 DEGs) pathway, while the DEGs in S + B vs S comparison are mainly enriched in starch and sucrose metabolism (7 DEGs), plant hormone signal transduction (6 DEGs), protein processing in endoplasmic reticulum (6 DEGs) (Table 1). In which, the pathway of plant hormone signal transduction, starch and sucrose metabolism, plant-pathogen interaction and amino sugar and nucleotide sugar metabolism had 2 common DEGs both in S vs CK and S + B vs S comparisons.

**Analysis of the most significantly expressed 100 DEGs**

By analyzing the 100 most significantly expressed DEGs of S vs CK and S + B vs S comparisons in the KEGG pathway

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Core DEGs analysis of S vs CK and S+B vs S comparisons in *G. uralensis*. A, Venn diagram of DEGs in G0 and G2. G0, DEGs of S vs CK; G2, DEGs of S+B vs S. B, The up-regulated and down-regulated number of core DEGs in S vs CK and S+B vs S comparisons. The number of DEGs is shown in the diagram. CK, control group; S, salt stress group; S+B, salt stress combined with G2 group.
The regulation of plant hormones in *G. uralensis* under salt stress by G2 inoculation

Through KEGG analyses of the first 20 pathways and the most significantly expressed 100 DEGs in S + B vs S comparison, we found that G2 significantly regulated plant hormone signal transduction in *G. uralensis* under salt stress, including IAA, ABA and JA. To verify the reliability of the expression level in plant hormone regulation from RNA-seq transcriptomes after treatment of G2 in *G. uralensis*, 5 genes influenced by salt or G2 were selected for quantitative real-time PCR (Table S4; Fig. S2). According to our statistics, the results of qRT-PCR were consistent with the transcriptome results (Fig. S2).

The KEGG map of plant hormone signal transduction in S vs CK and S + B vs S comparisons were shown in Figure 6. The figure showed that salt stress affected signal transduction pathways of six kinds of plant hormones (IAA, ABA, JA,
CTKs, ET and GAs, among which, only some DEGs encoding small auxin-up RNA (SAUR) family protein in IAA signal transduction pathway and some DEGs encoding EIN3-binding F-box protein in ET signal transduction pathway were up-regulated, while the other DEGs were down-regulated. The figure also showed that G2 mainly regulated signal transduction pathways of IAA, ABA and JA under salt stress. In order to clarify the regulation mechanism of G2 on plant hormones under salt stress, we mainly analyzed the three hormones IAA, ABA and JA (Table 2 and Figure 7).

As shown in Figure 7, the regulation of plant hormones by G2 under salt stress can be divided into three steps: biosynthesis, signal transduction and catabolism. For IAA, its biosynthesis and catabolism were not affected by salt stress or G2 inoculation. In IAA signal transduction, genes related to auxin influx carrier (AUX1), auxin/indole acetic acid repressors (AUX/IAA), IAA responsive Gretchen Hagen 3 (GH3) gene family and SAUR family protein are regulated by salt stress or G2 inoculation. In which, the DEGs encoding AUX/IAA, GH3 and some DEGs encoding SAUR were down-regulated, while others encoding SAUR were up-regulated in S vs CK comparison. In S + B vs S comparison, the DEG (Glyur000017s00002448) encoding SAUR family protein was down-regulated, while the DEG (Glyur000465s00024387) encoding IAA transporter-like protein 2 (LAX2) and the DEG (Glyur000111s0000702) encoding AUX/IAA9 were up-regulated in S + B vs S comparison (Table 2).

For ABA, its biosynthesis, signal transduction and catabolism were regulated by salt stress or G2 (Figure 7). The proteins including 9-cis-epoxy-carotenoid dioxygenase (NCED) in ABA biosynthesis, abscisic acid receptor (PYL), protein phosphatase 2C (PP2C) and serine/threonine-protein kinase (SAPK) in ABA signal transduction, and abscisic acid 8'-hydroxylase (CYP707A) in ABA catabolism were regulated in G. uralensis by salt or G2. In ABA biosynthesis, the DEG (Glyur000426s00022723) encoding NCED was up-regulated in S + B vs S comparison. In ABA signal transduction, the DEGs encoding PYL (Glyur000150s00014575 and Glyur007908s00046209) and PP2C (Glyur000061s00006499) were down-regulated in S vs CK comparison, while the DEG (Glyur002238s00033932) encoding SAPK was down-regulated and the DEG (Glyur000537s00024608) encoding PP2C was up-regulated in S + B vs S comparison. In ABA catabolism, two DEGs (Glyur000340s00024368 and Glyur000349s00021101) encoding CYP707A were down-regulated in S vs CK comparison, while the DEG (Glyur000402s00033324) encoding CYP707A was up-regulated in S + B vs S comparison (Table 2).

For JA, only allene oxide cyclase (AOC) in JA biosynthesis and JA-amido synthetase JAR1, coronatine-insensitive protein 1 (COI1), jasmonate ZIM domain-containing protein (JAZ) and transcription factor MYC2 in JA signal transduction were regulated by salt stress or G2 (Figure 7). In JA biosynthesis, the DEG (Glyur000815s00035014) encoding AOC was up-regulated in S + B vs S comparison.
In JA signal transduction, DEGs encoding JAR1 (Glyur000194s00016345), COI1 (Glyur001133s00027787), JAZ (Glyur000047s00003994, Glyur000475s00003994, Glyur000178s00013197 and Glyur001007s00026105) and MYC2 (Glyur003343s00045195) were down-regulated in S vs CK comparison, while a DEG (Glyur003343s00045195) encoding MYC2 was up-regulated in S + B vs S comparison (Table 2).

The regulation of transcription factors (TFs) in *G. uralensis* under salt stress by G2 inoculation

This study predicted a total of 5,449 regulatory genes from the new transcripts obtained. In Figure 8, DEGs encoding TFs were identified and mainly enriched in AP2 (APETALA2)/ERF (ethylene-responsive factor) (22 DEGs), NAC (NAM, ATAF1/2, CUC1/2) (19 DEGs), WRKY (16 DEGs), MYB (12 DEGs), MYB-related (11 DEGs) and GRAS (8 DEGs) subfamilies in S vs CK comparison. In the above six subfamilies, two DEGs in MYB subfamily and two genes in MYB-related subfamily were up-regulated, while other DEGs were down-regulated. DEGs encoding TFs in S + B vs S set were mainly enriched in GRAS (8 DEGs), C2H2 (3 DEGs), NF-YB (2 DEGs), Trihelix (2 DEGs), AP2/ERF-ERF (2 DEGs) and bHLH (basic helix-loop-helix protein) (2 DEGs). In the above six subfamilies, most DEGs enriched in GRAS subfamily, in which, six DEGs were down-regulated and two DEGs were up-regulated. All three DEGs in C2H2 subfamily were down-regulated and all two DEGs in NF-YB subfamily and two DEGs in Trihelix subfamily were up-regulated. Both in AP2/ERF-ERF and bHLH subfamily, a DEG was up-regulated, while the other one was down-regulated.

Discussion

Many plants such as soybean, maize, tomato and rice can benefit from endophytes, resulting in enhanced plant growth and alleviated salt stress (Shahzad et al. 2017; Lubna et al. 2018; Win et al. 2018; Gul et al. 2019). In our previous study, endophytic *B. cereus* G2 improved salt tolerance and thus promoted the growth of *G. uralensis* seedlings, but the mechanisms are not clear especially at the molecular level.
Given this situation, whole transcriptome analysis in *G. uralensis* with or without G2 under salt stress was performed using RNAseq sequencing. KEGG analysis revealed that plant hormone signal transduction pathway not only was the most enriched pathway but also contained the largest number of highly expressed DEGs in S + B vs S comparison, thereby we mainly analyzed the DEGs related to plant hormone biosynthesis, signal transduction and catabolism. Moreover, G2 mainly regulated IAA, ABA and JA in our results, so these three phytohormones are mainly discussed. Plant hormones are regarded as essential endogenous molecules involved in regulating plant growth and tolerance or susceptibility to diverse stresses including salinity stress (Ryu and Cho 2015). In IAA biosynthesis, tryptophan aminotransferase of *Arabidopsis* (TAA) is involved in the conversion of tryptophan to indole-3-pyruvate (IPA), and YUC (IAA biosynthetic enzyme indole-3-pyruvate mono-oxygenase YUCCA) is involved in IPA to IAA (Mashiguchi et al. 2011; Zhao 2014). In our study, genes encoding YUC and TAA were not differentially regulated by salt stress or G2, which suggested that G2 had little effect on IAA biosynthesis in *G. uralensis*. In IAA signal transduction, IAA can enter cells by diffusion when protonated or through the Auxin transporter carrier1/auxin transporter-like proteins (AUX1/LAX) in influx carriers (Naser and Shani 2016). In our study, G2 up-regulated the DEG encoding Auxin transporter-like protein 2 (LAX2) under salt stress, which indicated that G2 could help IAA to enter cells and further promote the absorption of IAA in *G. uralensis*. Moreover, IAA-responsive genes fit into three major classes: the so-called AUX/IAA, the GH3, and the SAUR gene families. Of which, AUX/IAAs, which function as transcriptional repressors of early IAA-responsive gene expression, negatively regulate IAA-mediated transscription by binding ARFs through conserved domains III and IV found in both types of proteins (Szemenyei et al. 2008; Hagen 2015). GH3 family genes cause IAA to bind to amino acids and regulate the feedback regulation of IAA, thus controlling the homeostasis of intracellular IAA (Chen et al. 2010). At low IAA levels, the expression of auxin-responsive genes is reduced or eliminated due to the interaction of dominant AUX/IAA repressors with ARF activators on the promoter of these genes. At higher IAA levels, the inhibitory effect of AUX/IAA protein on ARF is then released, allowing it to activate the regulation of downstream genes (Salehin et al. 2015). The DEG encoding AUX/IAA and the GH3 were down-regulated in S vs CK comparison, while the DEG encoding AUX/IAA was up-regulated in S + B vs S comparison. Based on the above results, it is speculated that the content of IAA in *G. uralensis* is low under salt stress, so AUX/IAA binds to ARF, thereby inhibiting ARF’s regulation of downstream DEGs encoding AUX/IAA proteins and the GH3 family, further disrupting IAA homeostasis. However, under the S + B treatment, the content of IAA in *G. uralensis* is elevated, and the inhibition effect of AUX/IAA protein on ARF is subsequently relieved and up-regulated the downstream DEG encoding AUX/IAA proteins. G2 had little effect on the GH3 gene under salt stress, which may be due to the homeostasis of IAA in *G. uralensis* cells has been controlled under S + B treatment and no further regulation is required. SAURs, as IAA responsive genes are key effector outputs of hormonal and environmental signals that regulate plant growth and development (Ren and Gray 2015). A previous study found that SAUR50 can promote cell elongation by inducing cell wall acidification (Mourik et al. 2017). The At12g46690 gene encoding a SAUR protein (SAUR32) was related to apical hook development (Park et al. 2007). In *Arabidopsis*, SAUR36 affects leaf senescence as a positive regulator (Hou et al. 2013). In our study, salt stress up-regulated the DEGs encoding SAUR50, but down-regulated the DEGs encoding SAUR32 and SAUR36, while G2 down-regulated a DEG encoding SAUR50 under salt stress, indicating that salt stress could promote cell elongation, affect apical hook development, and inhibit leaf senescence, while G2 could inhibit cell elongation, which may be a measure of G2 to protect *G. uralensis* cells under salt stress.

The phytohormone ABA played a key role in the activation of plant cellular adaptation to salinity and had a pivotal function as a growth inhibitor (Golldack et al. 2014). Salt stress generally caused osmotic stress and water deficit, increasing the production of ABA in plants. The accumulation of ABA can mitigate the inhibitory effect of salinity on photosynthesis, growth, and translocation of assimilates (Gupta and Huang 2014). The elevated ABA hormone helped plants to acclimate under lower water availability by closing stomata and accumulating numerous proteins and osmoprotectants for osmotic adjustment (Ryu and Cho 2015). A previous study showed that salt stress rapidly
activated genes encoding the protein related to ABA biosynthesis, such as zeaxanthin oxidase, NCED, ABA aldehyde oxidase and molybdenum cofactor sulfatase (Ryu and Cho 2015). In our study, the DEG encoding NCED was up-regulated by G2 in *G. uralensis* under salt stress, which may help plants produce ABA to cope with external stimuli to a greater extent under salt stress. The rapid production of ABA is necessary to deal with salt stress. However, when stress is relieved, the same rapid ABA catabolism is also essential (Zhang et al. 2006). CYP707A is related to ABA catabolic metabolism (Chen et al. 2019). In our results, salt stress down-regulated CYP707A1 and CYP707A3, while G2 up-regulated CYP707A2 under salt stress, indicating that G2 not only could help *G. uralensis* seedlings producing continuously accumulated ABA to alleviate the damage of salt stress but also could catabolize timely the excessive ABA when the harmful effect induced by salt was alleviated. In ABA signal transduction, the cytoplasmic ABA receptor PYR/PYLs/RCARs bind to PP2C to inhibit PP2C activity, and thus activates SnRK2. The active SnRK2 further activates the transcription factor ABFs/AREB1 to initiate downstream gene expression. A previous study found that ABA inhibited PYR1/PYL/RCAR ABA receptors and PP2C co-receptors, thereby inhibiting seed germination and seedling growth under adverse conditions (Wang et al. 2020). In our study, the DEGs encoding ABA receptor PYL and PP2C were down-regulated under salt stress, which may further inhibit seed germination and seedling growth in *G. uralensis*. After inoculation of G2 under salt stress, the DEG encoding PP2C was up-regulated and the DEG encoding serine/threonine-protein kinase SnRK2 was down-regulated, indicating that G2 activate PP2C activity, thereby inhibiting SnRK2 activity, and then terminate ABA signaling. Continuous activation of ABA signals generally led to an overreaction in plants, which is not conducive to plant growth, while G2 can help *G. uralensis* stop ABA signals to maintain plant growth under salt stress.

Over expressing AOC1 enhanced tolerance to salt stress by activating JA biosynthesis in transgenic *Arabidopsis* plants (Qiu et al. 2014). In our study, G2 up-regulated the DEG encoding AOC under salt stress, which promoted the biosynthesis of JA, thereby possibly increasing *G. uralensis* salt tolerance. In IAA signal transduction, salt stress down-regulated the DEG encoding protein JA-amido synthetase JAR1 that related to JA biosynthesis, thus impairing JA biosynthesis of *G. uralensis* under salt stress condition. Moreover, JA regulates plant development and defense responses through receptor proteins SCF COI1 and JAZ transcription factors (Kazan and Manners 2012). In our study, salt stress down-regulated the DEGs encoding key enzymes
COI1 and JAZ in JA signal transduction, thus possibly inhibiting plant growth. MYC2, as a key component linking the ABA- and JA-mediated salinity response pathways (Zhao et al. 2014), was down-regulated in *G. uralensis* seedlings under salt stress, while G2 up-regulated MYC2 under salt stress which may be one of the strategies that increasing *G. uralensis* salt tolerance by G2.

TFs are regulatory proteins that play important roles in plant growth and development and the biosynthesis of secondary metabolites, and help to regulate plant responses to adverse conditions (Erpen et al. 2018; Zhang et al. 2020). Through the analysis of TFs (Figure 8), salt stress mainly affected the TF subfamilies of AP2/ERF-ERF, NAC, WRKY, MYB, MYB-related and GRAS, but G2 mainly regulated GRAS, C2H2, NF-YB, Trihelix, AP2/ERF-ERF and bHLH subfamilies in *G. uralensis* under salt stress. Previous studies showed that all TFs mentioned above were related to plant response to salt stress (Huang et al. 2007; Fang et al. 2010; Golldack et al. 2014; Zhang et al. 2015; Hanin et al. 2016; Zhang et al. 2020). Specifically, AP2/ERF TFs are involved in plant growth and development, especially in response to biological or abiotic stresses in plants (Erpen et al. 2018). Moreover, AP2/ERF TFs are not only related to ET biosynthesis (Han et al. 2020; Zhang and Huang 2010), but are involved in the transcription of ABA biosynthesis genes (Yoon et al. 2020). In our study, salt stress down-regulated the genes encoding AP2/ERF family, while G2 up-regulated a gene and down-regulated a gene encoding AP2/ERF family, thus possibly improving *G. uralensis* salt tolerance by affecting ET and ABA biosynthesis. The MYB family regulated stomatal movement in *Arabidopsis thaliana* and rice through ABA signaling cascades, thereby regulating water loss (Chen et al. 2006; Zhao et al. 2018). The MYB family also can mediate ET biosynthesis to enhance plant salt tolerance (Xu et al. 2019), and overexpression of MYB family genes can improve plant disease resistance by regulating SA accumulation and JA signaling pathways (Raffaele et al. 2006; Erpen et al. 2018). In our results, salt stress
down-regulated almost all DEGs encoding the MYB family, while G2 up-regulated the only DEG encoding MYB family, thus possibly improving *G. uralensis* salt tolerance by regulating the signal transduction of ABA and IAA and the biosynthesis of ET and SA. The bHLH family is related to ABA and regulating the signal transduction of ABA and JA and the biosynthesis of ET and SA. The bHLH family is related to ABA and lating the signal transduction of ABA and JA and the biosyn-
thetic possibly improving while G2 up-regulated the only DEG encoding MYB family, down-regulated almost all DEGs encoding the MYB family, indicating that G2 could improve *G. uralensis* salt tolerance by regulating ABA and JA signal transduction. GRAS family is plant-specific TFs that play a variety of roles in plant develop-
omental and stress responses. Overexpression of SIGGRAS7 belong to the GRAS family led to the down-regulation of many genes related to IAA and GA in tomato seedlings (Habib et al. 2019). In our study, salt stress down-regulated all DEGs encoding GRAS, while G2 up-regulated 2 DEGs and down-regulated 6 DEGs encoding GRAS under salt stress in *G. uralensis*, indicating that G2 affected IAA-related genes maybe by regulating GRAS TFs. NAC activity was closely related to ABA-induced leaf senescence in rice (Sakuraba et al. 2020). WRKY can increase plant tolerance by reducing sensitivity to ABA transcript levels and ABA-inducible genes (Zhao et al. 2020). In our results, salt stress down-regulated all DEGs belong to NAC and WRKY, which may affect ABA signal transduction. Nuclear factor Y (NF-Y) is a highly conserved TF comprising NF-YA, NF-YB and NF-
YC subunits. Among these, TANF-YB3, as a NF-YB gene in wheat (*Triticum aestivum*), regulated drought response through regulating ABA-related pathways (Yang et al. 2017). In our study, G2 up-regulated 2 genes belong to the NF-YB family, thus possibly affecting the ABA-related pathway.

**Conclusion**

Through transcriptome analysis, we found that the regulation of plant hormone may be one of the important strategies that G2 improve *G. uralensis* salt tolerance. Multiple plant hormones biosynthesis, signal transduction and catabolism are regulated by *Bacillus cereus* G2 in *G. uralensis* subjected to salt stress, especially in IAA, ABA and JA. Specifically, G2 up-regulated the DEGs encoding AUX1 and AUX/IAA in IAA signal transduction; G2 up-regulated the DEGs encoding NCED, PP2C and CYP707A in ABA biosynthesis, signal transduction and catabolism, respectively; G2 up-regulated the DEGs encoding AOC and MYC2 in JA biosynthesis and signal transduction in *G. uralensis* under salt stress. In which, the effect of G2 on ABA biosynthesis in *G. uralensis* may be related to the regulation of AP2/ERF. The effect of G2 on IAA signal transduction may be related to the regulation of GRAS, the effect of G2 on ABA signal transduction may be related to the regulation of MYB, bHLH and NF-YB, and the JA signal transduction in *G. uralensis* may be related to the regulation of MYB and bHLH. The information obtained in this study provides a new perspective for studying plant hormone-related pathways and genes involved in the G2 regulation of *G. uralensis* salt tolerance.
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X Xiao performed experiment and wrote the manuscript; QL Wang made the tables; DY Lang performed the experiment; YK Chu modified the language; Y Zhang performed the experiment; LJ Zhou collected the literatures. XH Zhang provided the ideas and revised the manuscript; All the authors agreed on the contents of the paper and post no conflicting interest.

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