BMC Plant Biology

RESEARCH Open Access

Effects of the plant growth-promoting rhizobacterium *Zobellella* sp. DQSA1 on alleviating salt-alkali stress in job's tears seedings and its growth-promoting mechanism

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Abstract

Plant probacteria as a sustainable microbial resource are crucial to plant, which not only promote plant growth but also increase the stress resistance of plants. In this study, whole-genome sequencing of *Zobellella* sp. DQSA1 was performed, and *Zobellella* sp. DQSA1 was applied to Job's tears seedings under salt-alkali stress. Whole-genome analysis revealed that *Zobellella* sp. DQSA1 can produce metabolites such as tryptophan, alpha-linolenic acid and other products through metabolism. In response to the action of *Zobellella* sp. DQSA1, the contents of jasmonic acid (JA) and indole-3-acetic acid (IAA) in the root system increased by 32.5% and 81.4% respectively, whereas the content of abscisic acid (ABA) decreased by 30.0%, and the contents of other endogenous hormones also significantly differed. Additionally, the physiological and biochemical indices related to growth and salinity demonstrated notable differences. Finally, sequencing analysis revealed that 57 differentially expressed genes (DEGs) were involved in 16 Gene Ontology (GO) pathways. Furthermore, the correlations between the contents of endogenous hormones and 57 DEGs were analyzed, and JA was found to be the most significantly correlated. These results provide a theoretical basis for further exploration of the functions and mechanisms of plant growth-promoting rhizobacteria (PGPR) under salt-alkali stress.

Keywords Job's tears, PGPR, Salt-alkali stress, Endogenous hormone, Differentially expressed genes

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Introduction

Salt-alkali stress is a prevalent abiotic stress that affects approximately 952.2 million hectares of land worldwide and is increasingly becoming a significant constraint on global crop production [64]. In Northeast China, more than 70% of the grasslands are alkaline. The inclusion of inorganic substances, such as neutral salts, sodium carbonate (Na_2CO_3), and sodium bicarbonate ($NaHCO_3$) in salt-alkali soils can increase soil environment pH, resulting in significant growth inhibition and crop damage [9]. Salt–alkali stress is more complex than neutral salt stress because it disrupts cellular pH homeostasis during crop growth, which can have negative effects on crop yield [41].

The diverse communities of microorganisms in the rhizosphere host are crucial to plants, not only providing nutrients but also protecting them from pathogens [13]; beneficial microorganisms are known as PGPRs, which promote plant growth, development, and stress tolerance. Rhizosphere microorganisms can directly affect plant growth, yield and quality ect. In addition, the microbiome can stimulate plant growth by producing phytohormones [27]. Several PGPRs have been studied in plants and found to increase plant tolerance to salt-alkali, drought, and heavy metal stresses, in addition to their growth-promoting activity [19, 54, 66]. Currently, the mechanism of microbial-plant interaction has become a hot topic. The exploration of salt-alkali-tolerant and plant-promoting bacterial strains can provide theoretical support for the recovery and reconstruction of salt-alkali land. Additionally, the development and utilization of microbial biotrophic bacteria provide a new direction for salt–alkali land management.

Zobellella sp. DQSA1 is a new strain of extremophile microorganism that was isolated from the Daqing salt-alkali land in China, which has prebiotic functions, such as providing iron-producing carriers, producing ACC deaminase, producing the phytohormone IAA, solubilizing phosphorus, and solubilizing potassium [69]. Meanwhile, *Zobellella* sp. DQSA1 is also a denitrifying bacterium. While there are currently no reports on plant promotion under adverse conditions by*Zobellella* sp. DQSA1, it has been reported that denitrifying bacteria such as *Pseudomonas* and *Alcaligenes* can promote plant growth under adverse conditions [15, 57]. Denitrifying bacteria have numerous applications in wastewater treatment and environmental purification [2], and their main mode of action is the degradation of nitrate [44]. Therefore, we speculate that *Zobellella* sp. DQSA1 converts ammonia into nitrite and nitrate that are more easily absorbed by plants through nitrification, and providing a key nitrogen source and promoting the nutrient supply of plants. In addition, the hydrogen ions were released by *Zobellella* sp. DQSA1 during the decomposition process to help to regulate soil pH, which can create a suitable root growth environment and work together with other microorganisms to balance the soil microbial community. The suitable environment can inhibit the reproduction of harmful pathogens and provide good growth conditions for healthy plants growth and development.

Job's tear (*Coix lacryma-jobi L.*) is an ancient graminaceous plant with high nutritional and medicinal value, which are used as a crop for both medicine and food in China. Owing to their versatility, they are known as the king of the world's graminaceous family [22]. As a medicinal herb, Job's tear fruit has the properties of being sweet, mild and heat-clearing, which can be used to treat neuralgia, inflammatory diseases, rheumatism, and osteoporosis, and as a diuretic to remove edema, strengthen the spleen, dispel dampness, and remove heat and pus [72]. As an edible raw material, its calorie is higher than rice and wheat, and is rich in fat, a variety of amino acids, vitamin B, vitamin B_2, Ca, P, Mg, and so on [74]. The crop with medicinal and food value, has been introduced into China's salt-alkali areas for cultivation. However, the growth of Job's tear grains is hindered in salt–alkali areas, resulting in seedling shortages, yield reduction and quality decline. Given the context of promoting sustainable development, agricultural microbial agents have been widely used.

In this work, we studied the physiology, growth parameters and transcriptome analysis of Job's tear seedlings in response to *Zobellella* sp. DQSA1 under salt-alkali stress. This study not only investigated the mechanism and response of *Zobellella* sp. DQSA1 to alleviate salt-alkali stress and promote its growth at physiological and molecular perspective but also provides some reference for the cultivation of Job's tears in other salt-alkali regions. Moreover, these findings provide a foundation for further exploration of the function and mechanism of action of PGPR under salt-alkali stress.

Materials and methods

Whole-genome sequence analysis of *Zobellella* Sp. DQSA1

Zobellella sp. DQSA1 is a new strain of extremophile microorganism that was isolated from the saline–alkali Na_2CO_3 type of Daqing salt-alkali land in China. Additionally, it has prebiotic functions, such as providing iron-producing carriers, producing ACC deaminase, producing the phytohormone IAA, solubilizing phosphorus, and solubilizing potassium [69].

The DNA samples that passed the *Zobellella* sp. DQSA1 assay were subjected to gel recovery and DNA library construction. Majorbio Biomedical Technologies (Shanghai, China) completed second- and third-generation genome sequencing via the Illumina noveseq-6000 and PacBio sequencing platforms. The genome was assembled via three generations of data. The original data

were self-corrected, and the initial genome assembly was performed via Falcon to obtain the consensus sequence. The gene sequencing data were subjected to KEGG and GO analyses, as well as other related analysis [10].

Plant material collection

"Yiliao No.5" of *Coix lacryma-jobi* L. varity was provided by the Plant and Microbial Interaction Research Group of Heilongjiang Bayi Agricultural University, Heilongjiang Province, China, was used as the test material in this study. The experiment was conducted indoors using Job's tear seeds, which were uniform in size, full, and unbroken. All test-treated seeds were soaked in water for 24 h, disinfected with 2% NaClO (v/v) for 10–15 min, and then rinsed with sterile distilled water 3–5 times. Seeds of consistent germination were transferred to Hoagland nutrient solution and incubated under standard room temperature conditions (25℃ and 12 h light–dark periods). Once the Job's tear seedlings reached the two-leaf-one-heart stage, salt-alkali buffer solution (Na_2CO_3:$NaHCO_3$ = 9:1) was added to the Hoagland nutrient mixture at a 1:1 ratio. The Job's tears seedlings were then transferred into the mixture for further cultivation. Four concentrations of salt-alkali buffer solution (10 mM, 20 mM, 30 mM, 40 mM) were used to screen for salt-alkali stress concentrations. The control (CK) consisted of a 1:1 mixture of water and Hoagland nutrient solution. Three replications of each treatment were conducted.

After screening for a suitable stress concentration, Job's tears seedlings grown to the two-leaf-one-heart stage were stressed at screened concentration for 48 h and then the growth promotion experiments were carried. The experiments were divided into four groups, namely, the control group (CK), the group receiving *Zobellella* sp. DQSA1 only (CK+), the salt-alkali stress group (SA), and the salt-alkali stress plus *Zobellella* sp. DQSA1 group (SA+). After *Zobellella* sp. DQSA1 was activated in LB solid medium with a pH of 8.5, single colonies were selected and transferred to LB liquid medium with the same pH. The mixture was then incubated on a shaker for 12 h at 28 ℃ and 170 rpm. From this culture, 400 mL of bacterial mixture was removed and centrifuged at 4℃ and 8000 rpm for 10 min. The supernatant was removed, and the concentration of 8.3×10^8 cfu/mL bacterial cells were resuspended in physiological saline (9 g NaCl in 1 L water) before being added to CK + and SA + for growth promotion experiments. After *Zobellella* sp. DQSA1 was added, samples were collected from each group at 0, 12, 24, 36, 48 and 60 h, and stored in -80 ℃ for the future use.

Measurement of growth indicators

The height, root length, and stem diameter of the Job's tears seedlings were measured via a Vernier caliper. The above-ground and underground parts of the Job's tears seedlings were dried at 105℃ for 30 min and then at 65℃ for 36 h. The average dry biomass above and below ground was recorded and calculated. Measurements were taken at three positions on the leaf (bottom, middle, and top) via a SPAD-502 chlorophyll meter. The average SPAD value for each leaf was calculated [26].

The root indicators of Job's tears seedlings were scanned via a root scanning device (PerfectionV-700Photo, Seiko Epson Corp., Japan), and the roots from each treatment were analyzed via WinRHIZO software (Regent Instruments Inc., Canada). Root scans were performed by gently rotating the root system with forceps to avoid overlapping lateral roots and to minimize errors. The root surface area, root volume, mean diameter and number of root tips were recorded carefully.

Measurement of physiological and biochemical indicators

The enzyme mixture was obtained through the following procedure: The enzyme was extracted from 0.5 g of leaves or roots in a precooled mortar and pestle, then 0.1 M phosphate buffer was added and the mixture was centrifuged for 15 min, which supernatant was utilized for the assessment of enzyme activity.an the enzyme extracts were prepared at a temperature of 4℃ [45].

Samples were collected at 0, 12, 24, 36, 48 and 60 h to measure the following indices. The concentration of H_2O_2 was determined by measuring its absorbance at 410 nm (Shams [56]). The O_2^- content was determined via the hydroxylamine oxidation method [51]. Superoxide dismutase (SOD) activity was determined via nitroblue tetrazolium [71]. Peroxidase (POD) activity was determined by guaiacol [1]. Catalase (CAT) activity was assayed and calculated by monitoring the initial rate of H_2O_2 disappearance at 240 nm [20]. The 2-thiobarbituric acid (TBA) method was used to determine the content of malondialdehyde (MDA) [8]. The free proline content was determined via the sulfosalicylic acid method [7]. The soluble protein content was determined via the Thomas Brilliant Blue G-250 staining method [53], which measurements were repeated three times.

Histochemical detection of hydrogen peroxide (H_2O_2) and superoxide anion radical (O_2^-)

At 36 h of treatment, leaves were collected via sterilized scissors to ensure clean cuts, and 5 cm of leaves were removed. To detect H_2O_2, the leaves were placed randomly into 10 mL test tubes containing 3,3′-diaminobenzidine tetrahydrochloride (1 mg/mL, pH 5.8) solution (Coolaber, Beijing, China), and the tubes were kept in the dark at 25℃ for 12 h. To detect O_2^-, the leaves were

randomly placed in test tubes containing 10 mL of 0.1% nitroblue tetrazolium solution (Coolaber, Beijing, China) in potassium phosphate buffer (10 mM, pH 6.8) and infiltrated for 24 h. The staining solution was discarded from the test tube, and 75% ethanol was added to clean it. The tube was then placed in a high-temperature water bath and boiled, and attention was given to replenishing 75% ethanol until the chlorophyll on the leaf blade was completely removed. This was indicated by the absence of a green color in the ethanol mixture in the test tube. Brown and blue spots were observed, indicating the deposition of H_2O_2 and O_2^-, respectively [51].

Endogenous hormone content determination

The contents of Zeatin (ZR), IAA, ABA, Brassinosteroids (BR), Gibberellins (GA), JA and Kinetin (KT) were determined via liquid chromatography–mass spectrometry (LC–MS). One gram of roots from different treatments was weighed accurately in a 50 mL centrifuge tube, and 10 mL of acetonitrile: water: formic acid (80:19:1) was added. The mixture was then vibrated for one minute, sonicated in an ice bath for 20 min, and extracted at -20℃ for 16 h. After this period, the contents of each hormone were determined. The extract was maintained at a temperature of -20℃ for a period of 16 h, which the concentration of each hormone was quantified. The detection conditions of LC were as follows: mobile phase 10 mmol/L ammonium acetate aqueous solution-methanol; gradient elution: 0–10 min, 85% -5% ammonium acetate aqueous solution; 10–11 min, 5% ammonium acetate aqueous solution; 11–12 min, 5 -85% ammonium acetate aqueous solution; 12–15 min, 85% ammonium acetate aqueous solution; the flow rate was 0.3 mL/min. Injection volume: 10 μL; the column temperature was 30℃. MS conditions were positive and negative ion ionization modes of the electrospray ionization source and the spray voltage was 3 kV. The sheath gas (N_2) flow rates were 20 and 15 L/min in positive and negative ion ionization modes, respectively. The auxiliary gas (N_2) flow rates were 10 and 5 Arbs in positive and negative ion ionization modes, respectively. The capillary temperatures for ion transport were 300 and 200℃, respectively. The collision gas is argon and the collision gas pressure is 0.1995 Pa. Selective reaction monitoring (SRM) mode was used for scanning [60]. The ethylene (ETH) content was quantified via gas chromatography. The sample was weighed and placed in a gas collection bottle. The collection time was recorded and 1 mL of gas was extracted and measured by a HITACHI 163 gas chromatograph at room temperature for 2 h. The carrier gas was nitrogen at a flow rate of 1 mL/min, the inlet temperature was 50℃, the column temperature was 50℃, and gas chromatography was performed on an Agilent HP–PLOT Q 30 m×0.53 mm0.4 pm gas chromatograph. The separation was conducted on an Agilent HP-PLOT Q 30 m×0.53 mm0.4 pm GC column with an FID detector at 200℃ and a retention time of 1.1 min [16].

Transcriptome sequencing analysis and qRT–PCR validation

The roots of Job's tear seedlings subjected to different treatments were collected to prepare RNA samples, which treatment consisted of three biological replicates. The samples were treated for 36 h and then immediately snap-frozen in liquid nitrogen and stored at -80℃. Total RNA was extracted from the roots of Job's tears seedling via a plant RNA extraction kit manufactured by Tiangen (Beijing, China). The concentration, purity, and integrity of the extracted RNA were evaluated via a spectrophotometer and an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) prior to cryopreservation. cDNA library construction and transcriptome sequencing were performed by Majorbio-Biopharm-Technology (Shanghai, China).

DESeq 2 software was selected for the analysis and the Salmon algorithm was employed to calculate FPKM values, which indicate the expression levels of individual genes. RSEM was used to compare the quality control sequencing data with the assembled transcriptome sequences via bowtie, after which the expression abundance of the genes/transcripts was estimated on the basis of the results of the comparison. Finally, FPKM was used to compare the differences in gene expression between different samples. To increase the reliability of the analysis, the false discovery rate (FDR) method was employed to adjust the p-value [6, 55]. The researchers subsequently selected DEGs that exhibited significant differences for further investigation. The threshold for selection was set at$|\mathrm{Log}_2\ \mathrm{FC}| > 5.5$, which was used to determine their enrichment in GO terms, which are available at http://geneontology.org/. To investigate the relationships and associations between the datasets, a Mantel test via R software (version R-4.0.5) was performed. Additionally, correlation and co-occurrence network analyses was conducted according to the Spearman method [48].

To validate the transcriptome sequencing results, we analyzed the expression levels of 16 single genes in different treatment groups via qRT–PCR. Actin was used as the internal reference gene. Primer-BLAST software was used to design primers specific to the unigene sequence, as shown in Table S1. RNA was extracted from the samples and reverse transcribed into cDNA for qRT–PCR analysis. The reaction system consisted of 20 μL, with 1 μL of cDNA, 10 μL of 2×SYBR Green qPCR master mix, and 0.4 μL each of forward and reverse primers. A Bio-Rad CFX96 Real-Time System (Bio-Rad, USA) was used to perform all qRT–PCR. The program consisted of predenaturation at 95℃ for 2 min, followed by denaturation

at 95℃ for 10 s for 40 cycles and annealing/extension at 58℃ for 30 s. The reaction system was 20 μL, with 1 μL of cDNA, 10 μL of 2×SYBR Green qPCR master mix, and 0.4 μL each of forward and reverse primers. The $2^{-\Delta\Delta Ct}$ method was used to calculate the expression levels of the analyzed genes [42].

Statistical analysis

A correlation test was performed via R version 4.3.0 to statistically evaluate the correlation between endogenous hormones and gene expression. All the data in the tables and figures are the means of at least three replicates and the results are expressed as the means ± standard deviations (SDs). The data were statistically analyzed via IBM SPSS Statistics 23 to test the significance of the differences.

Results

Relevant metabolic pathways in the whole genome of *Zobellella* Sp. DQSA1

Whole-genome sequencing was conducted on *Zobellella* sp. DQSA1. The genome size of *Zobellella* sp. DQSA1 was 4,201,419 base pairs and the genome type was cyclic, which average content of GCs was 61.14% and the number of encoded genes was 3,801, including 98 tRNA genes and 25 rRNA genes (Fig. S1). KEGG analysis of the whole genome of *Zobellella* sp. DQSA1 revealed the related to genes expression of metabolism pathways such as histidine metabolism; glycine, serine and threonine metabolism; and tryptophan metabolism in the metabolism classification (Table 1). The metabolites produced through these metabolic pathways play important regulatory roles in plant growth promotion and stress tolerance.

Effects of *Zobellella* Sp. DQSA1 on the growth of job's tear seedlings

When Job's tears seedlings reached the two-leaf-one-heart stage, which were treated with a mixture of salt-alkali buffer and Hoagland nutrient solution at various concentrations. At 30 mM concentration, the Job's tears seedlings wilted but did not die (Fig. S2). Thus, the salt-alkali stress concentration was determined to be 30 mM.

Following the screening of the most suitable stress concentration, *Zobellella* sp. DQSA1 was added to Job's tear seedlings that had been stressed for 48 h to conduct growth promotion experiments. The results demonstrated that the addition of *Zobellella* sp. DQSA1 had a growth-promoting effect on the growth of Job's tears seedlings. (Fig. 1A). Compared with CK group, the fresh leaf weight of the CK + treated plants increased by 21.22%, the root length decreased by 15.42%, the stem thickness and chlorophyll content increased by 29.15% and 34.89% respectively. Compared with SA, SA + significantly increased all the growth indices, with 64.00% and 44.38% increases in the dry and fresh weight of leaves, 33.89% and 23.67% increases in the dry and fresh weight of roots, 19.80% increases in plant height, 33.79% increases in root length, 32.66% increases in stem thickness and 38.00% increases in chlorophyll content (Fig. 1B-I).

The root systems of the different treatment groups were scanned via a root scanner and were found to be significantly different (Fig. S3). Compared with CK treatment, the addition of *Zobellella* sp. DQSA1 resulted in significant differences in all the root indicators, which surface area, mean diameter, and number of root tips were increased by 59.19%, 63.46%, and 64.57% respectively, and the volume decreased by 33.42% in CK + group. Compared with SA treatment, all four indices were significantly increased by 39.30%, 15.85%, 114.61%, and 139.15% respectively in SA + group (Fig. 1J-M).

Effects of *Zobellella* Sp. DQSA1 on the physiological and biochemical parameters of job's tear seedlings

The antioxidant enzyme activities showed the trend of rising firstly and then decreasing in SA and SA + treatment

Table 1 Relevant metabolic pathways in the whole genome of *Zobellella* sp. DQSA1

First Category	Second Category	Third Category
Metabolism	Amino acid metabolism	Glycine, serine and threonine metabolism
Metabolism	Amino acid metabolism	Histidine metabolism
Metabolism	Amino acid metabolism	Alanine, aspartate and glutamate metabolism
Metabolism	Amino acid metabolism	Tryptophan metabolism
Metabolism	Amino acid metabolism	Lysine degradation
Metabolism	Amino acid metabolism	Lysine biosynthesis
Metabolism	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis
Metabolism	Amino acid metabolism	Cysteine and methionine metabolism
Metabolism	Amino acid metabolism	Arginine and proline metabolism
Metabolism	Amino acid metabolism	Tyrosine metabolism
Metabolism	Amino acid metabolism	Phenylalanine metabolism
Metabolism	Lipid metabolism	alpha-Linolenic acid metabolism

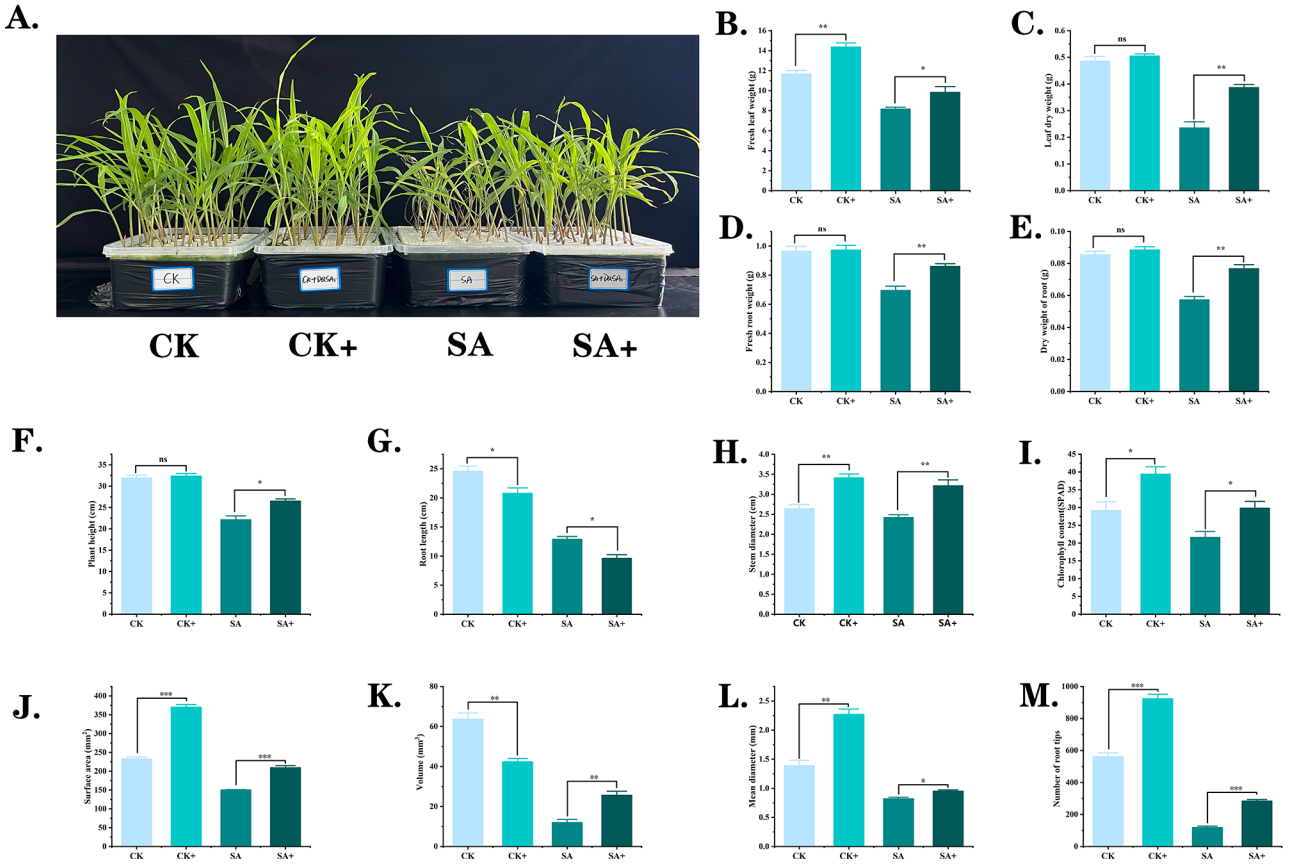


Fig. 1 The Job's tears seedlings growing situation and growth indexes of different treatment group. **A**: Growth phenotype, **B**: Fresh leaf weight, **C**: Leaf dry weight, **D**: Fresh root weight, **E**: Dry weight of root, **F**: Plant height, **G**: Root length, **H**: Stem diameter, **I**: Chlorophyll content, **J**: Surface area of root, **K**: Volume of root, **L**: Mean diameter of root, **M**: Number of root tips increasing. Data were analyzed using SPSS with t-tests; asterisks above indicate significant differences between the respective values, **P* < 0.05, ***P* < 0.01, ***P* < 0.001. All statistically analyzed data have three biological replicates

groups. At 36 h of treatment, the activities of CAT and POD of leaves increased by 40.0% and 13.4% respectively, in SA + group compared with SA group. In contrast, the activity of SOD decreased by 11.9%. At 48 h, the activity of SOD increased in the SA + group, reaching its maximum and being greater than that of the SA group at 36 h. In the root system, CAT activity, SOD activity and POD activity increased by 8.9%, 9.2% and 2.4% respectively, in SA + group compared with SA group. Additionally, there was a significant increase in the activities of antioxidant enzymes in the leaf and root systems in the CK + group compared with those in the CK group (Fig. 2A-F).

The results presented in Fig. 2G-L demonstrate that the amount of osmoregulatory substances also increased following the administration of *Zobellella* sp. DQSA1. At 36 h of treatment, the soluble sugar, soluble protein, and proline contents increased by 45.5%, 12.1%, and 38.9% respectively in the leaves and also increased by 23.6%, 4.1%, and 45.1% respectively in the roots of the SA + group compared with SA group. The difference between the CK group and the CK + group was not significant.

As shown in Fig. 2M-R, the contents of H_2O_2 and O_2^- in leaves and roots of each treatment group increased with time. Compared with SA group, the content of H_2O_2 and O_2^- in leaves of SA + group decreased by 17.7% and 5.1% respectively at 36 h, and decreased by 24.9% and 28.4% respectively in roots. At 60 h, the content of H_2O_2 and O_2^- in leaves of SA + group decreased by 16.2% and 19.5% respectively, and decreased by 31.9% and 15.9% respectively in roots. The difference between CK group and CK + group was not significant. Compared with SA group, MDA content in leaves of SA + group had no significant difference at 36 h, but decreased by 15.7% in roots. At 60 h, MDA content was different in leaves and roots, which decreased by 25.7% and 24.3%, respectively, and there was no significant change in MDA content of CK and CK + groups.

Histochemical localization of H_2O_2 and O_2^-

The leaves of the plants in the CK and CK + groups presented no or only weak staining for H_2O_2 and O_2^- deposition, while the leaves of the plants staining showed a significant difference in the leaves of the plants in the SA

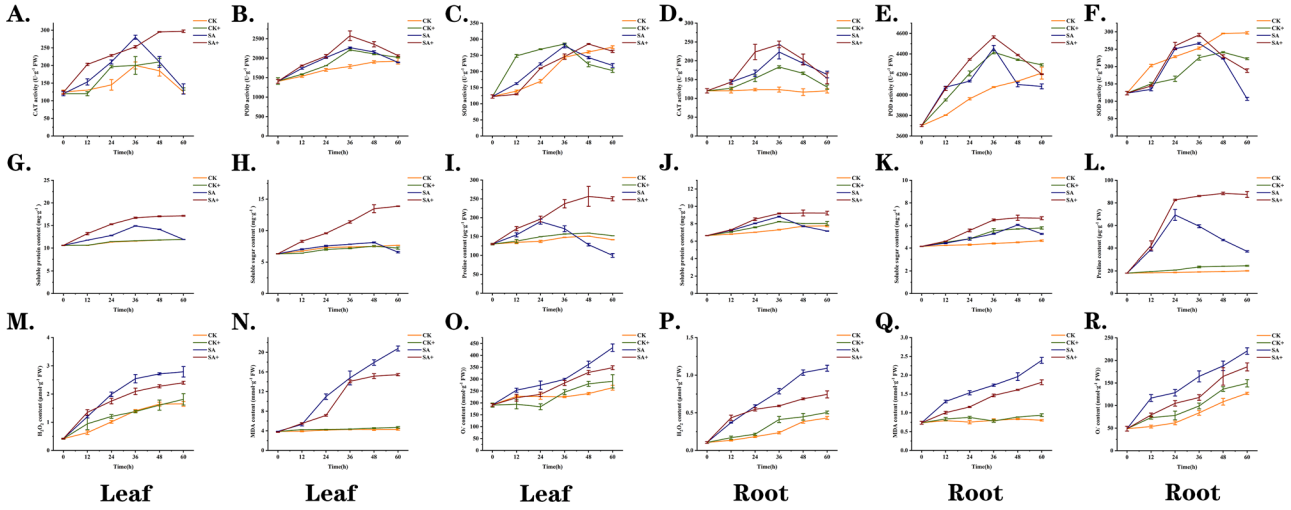


Fig. 2 Physiological and biochemical indicators in roots and leaves of Job's tears seedlings at different times under different treatment groups. **A**: CAT activity in leaves, **B**: POD activity in leaves, **C**: SOD activity in leaves, **D**: CAT activity in roots, **E**: POD activity in roots, **F**: SOD activity in roots, **G**: Soluble sugars content in leaves, **H**: Soluble proteins in leaves, I: Proline contents in leaves, **J**: Soluble sugars content in roots, **K**: Soluble proteins in roots, **L**: Proline contents in roots, **M**: H_2O_2 content in leaves, **N**: MDA content in leaves, **O**: O_2^- contents in leaves, **P**: H_2O_2 content in roots, **Q**: MDA content in roots, **R**: O_2^- contents in roots

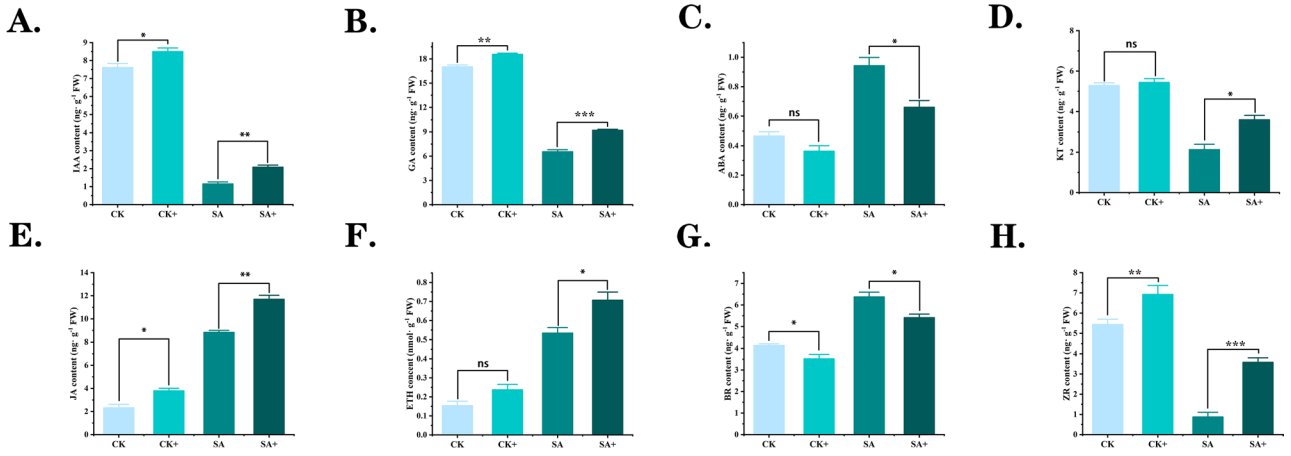


Fig. 3 Endogenous hormone contents in the root system of Job's tears seedlings under different treatments. **A**: IAA, **B**: GA, **C**: ABA, **D**: KT, **E**: JA, **F**: ETH, **G**: BR, **H**: ZR

and SA + groups, which the intensity of H_2O_2 (brown) and O_2^- (blue) deposition was lower in the leaves of the SA + group than in those of the SA group (Fig. S4).

Changes in endogenous hormones in job's tear seedlings

The impact of *Zobellella* sp. DQSA1 on the root system was more pronounced, with related enzyme activities reaching their maximum value at 36 h. Consequently, the contents of IAA, GA, ABA, KT, JA, ETH, BR, and ZR were determined comprehensively in Job's tear seedlings root for 36 h.The results demonstrated that the contents of IAA, GA, JA, and ZR were markedly greater in the CK group than in the CK + group, with increases of 11.7%, 9.1%, 64.5% and 27.4%, respectively. Conversely, the content of BR was lower, and no significant difference was detected in the contents of ABA, KT, and ETH. In the SA + group, the contents of IAA, GA, KT, JA, ETH, and ZR increased by 81.4%, 40.8%, 69.4%, 32.5%, 32.5% and 311%, respectively, and the contents of ABA and BR decreased by 30.0% and 15.1%, respectively (Fig. 3), which incicated a significant difference compared with SA group The results also showed that the growth-promoting effect of *Zobellella* sp. DQSA1 on Job's tear seedlings was more pronounced under salt-alkali conditions.

The screening and analyzing of key DEGs

Samples of Job's tear seedling root systems at 36 h were subjected to RNA-Seq analysis with three biological replicates. A total of 11,206 DEGs were identified, with 3,271 up-regulated and 7,935 down-regulated genes in

the CK_DQSA1 (CK+) vs. CK comparison. Similarly, 83,130 DEGs were observed, with 61,765 up-regulated and 21,365 down-regulated genes in the SA vs. CK comparison. Finally, 84,945 DEGs were found, with 39,709 up-regulated and 45,236 down-regulated genes in the SA_DQSA1 (SA+) vs. SA comparison. To identify the key DEGs, we employed UpSet and flower diagrams to summarize the number of DEGs in the three different groups. In the CK_DQSA1 vs. CK group and the SA_DQSA1 vs. SA group, there were 520 up-regulated DEGs and 5109 down-regulated DEGs, respectively (Fig. S5). To investigate the promotional effect of *Zobellella* sp. DQSA1 on Job's tear seedlings, DEGs that were expressed in both the CK_DQSA1 vs. CK groups and the SA_DQSA1 vs. SA groups with large differences in expression ($|Log_2 FC| > 5.5$) were selected for analysis, and a total of 173 eligible DEGs were identified (Table S2).

Functional annotation analysis of DEGs

The 173 significant DEGs were categorized into three distinct GO classes, encompassing biological processes, cellular components, and molecular functions. Among these genes, only 113 genes were annotated, as depicted in Fig. 4A. In biological process annotation, including the peptide metabolic process (GO:0006518), cellular amide metabolic process (GO:0043603), translation (GO:0006412), peptide biosynthetic process (GO:0043043), cellular nitrogen compound biosynthetic process (GO:0044271), macromolecule biosynthetic process (GO:0009059), amide biosynthetic process (GO:0043604), organonitrogen compound biosynthetic process (GO:1901566), and cellular macromolecule biosynthetic process (GO:0034645). In cellular component category, including the ribonucleoprotein complex (GO:1990904), ribosome (GO:0005840), non-membrane-bounded organelle (GO:0043228) and intracellular non-membrane-bounded organelle (GO:0043232). Within the molecular function category, three enriched GO terms stood out, namely structural constituent of ribosome (GO:0003735), mRNA binding (GO:0003729) and structural molecule activity (GO:0005198). In total, 57 DEGs demonstrated significant enrichment in 16 distinct GO pathways. Among these, thirty-two DEGs were notably enriched in GO:0006518 (peptide metabolic process), thirty-two DEGs in GO:0043603 (cellular amide metabolic process), thirty-one DEGs in GO:0006412 (translation), thirty-one DEGs in GO:0043043 (peptide biosynthetic process), thirty-six DEGs in GO:0044271 (cellular nitrogen compound biosynthetic process), thirty-one DEGs in GO:0009059 (macromolecule biosynthetic process), thirty-one DEGs in GO:0043604 (amide biosynthetic process), thirty-nine DEGs in GO:1,901,566 (organonitrogen compound biosynthetic process), thirty-one DEGs in GO:0034645 (cellular macromolecule biosynthetic process), thirty-six DEGs in GO:1,990,904 (ribonucleoprotein complex), twenty-nine DEGs in GO:0005840 (ribosome), thirty-four DEGs in GO:0043228 (non-membrane-bounded organelle), thirty-four DEGs in GO:0043232 (intracellular non-membrane-bounded organelle), thirty-two DEGs in GO:0003735 (structural constituent of ribosome), nineteen DEGs in GO:0003729 (mRNA binding) and thirty-eight DEGs in GO:0005198 (structural molecule activity) (Fig. 4B). In summary, we identified 57 key DEGs that regulate 16 different GO pathways in response to *Zobellella* sp. DQSA1.

Association analysis between key DEGs and endogenous hormones

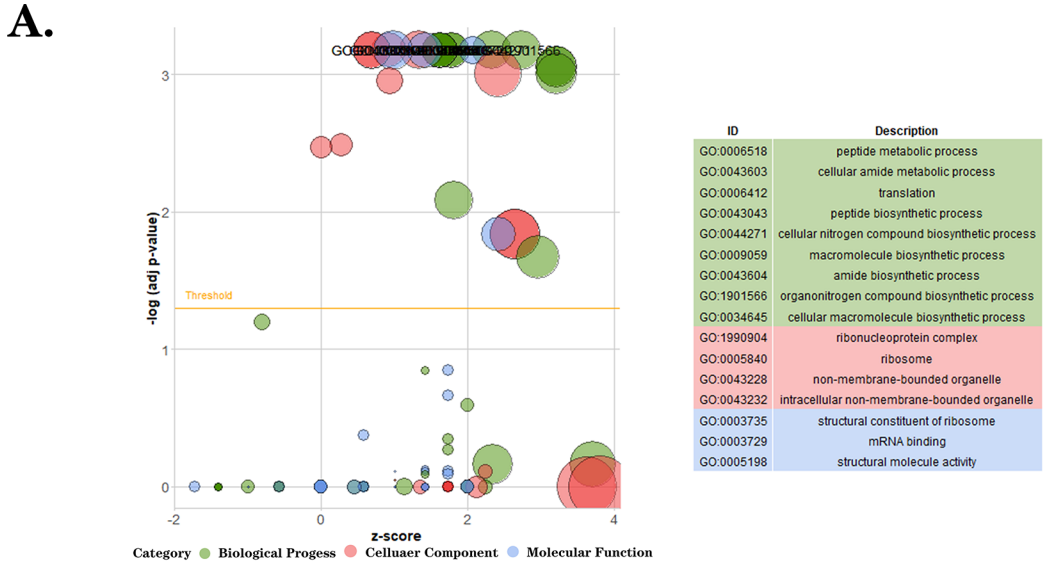
To analyze the associations among the 57 key DEGs of GO terms and endogenous hormone contents, the Mantel correlation test was employed. Given that the endogenous hormone content exhibited greater variability in the SA and SA + groups, these two groups were subjected to analysis. The results revealed a highly significant correlation between JA content and the BP, CC, and MF categories, as well as a significant correlation between ABA content and IAA content (Fig. 5A). Furthermore, we observed that 21 DEGs were significantly negatively correlated with JA content, 21 DEGs were significantly positively correlated with ABA content, 36 DEGs were significantly positively correlated with KT content, 36 DEGs were significantly negatively correlated with BR content, 21 DEGs were significantly negatively correlated with IAA content, and 21 DEGs were significantly negatively correlated with ETH content (Fig. 5B). Comprehensively, the correlation between endogenous hormones and key DEGs, between GO terms and key DEGs was seen Fig. 5C.

KEGG analysis of JA generation and regulation

The above analyses revealed that the DEGs correlated most significantly with JA. Therefore, the pathway involved in the generation and regulation of JA was analyzed. The KEGG metabolic pathway indicates that JA is synthesized by α-linolenic acid metabolism, in which lipoxygenase (EC: 1.13.11.12), hydroperoxide dehydratase (EC: 4.2.1.92) and allene oxide cyclase (EC: 5.3.99.6) are significantly up-regulated. In the plant hormone signal transduction pathway, jasmonic acid-amino synthetase (*JAR1*), coronatine-insensitive protein 1 (*COI1*), jasmonate ZIM domain-containing protein (*JAZ*), and the transcription factor MYC2 (*MYC2*) were significantly up-regulated (Fig. 6).

Validation of DEGs by qRT–PCR

To verify the accuracy and reproducibility of the RNA-seq results, a total of 16 genes were randomly selected



ID	Description
GO:0006518	peptide metabolic process
GO:0043603	cellular amide metabolic process
GO:0006412	translation
GO:0043043	peptide biosynthetic process
GO:0044271	cellular nitrogen compound biosynthetic process
GO:0009059	macromolecule biosynthetic process
GO:0043604	amide biosynthetic process
GO:1901566	organonitrogen compound biosynthetic process
GO:0034645	cellular macromolecule biosynthetic process
GO:1990904	ribonucleoprotein complex
GO:0005840	ribosome
GO:0043228	non-membrane-bounded organelle
GO:0043232	intracellular non-membrane-bounded organelle
GO:0003735	structural constituent of ribosome
GO:0003729	mRNA binding
GO:0005198	structural molecule activity

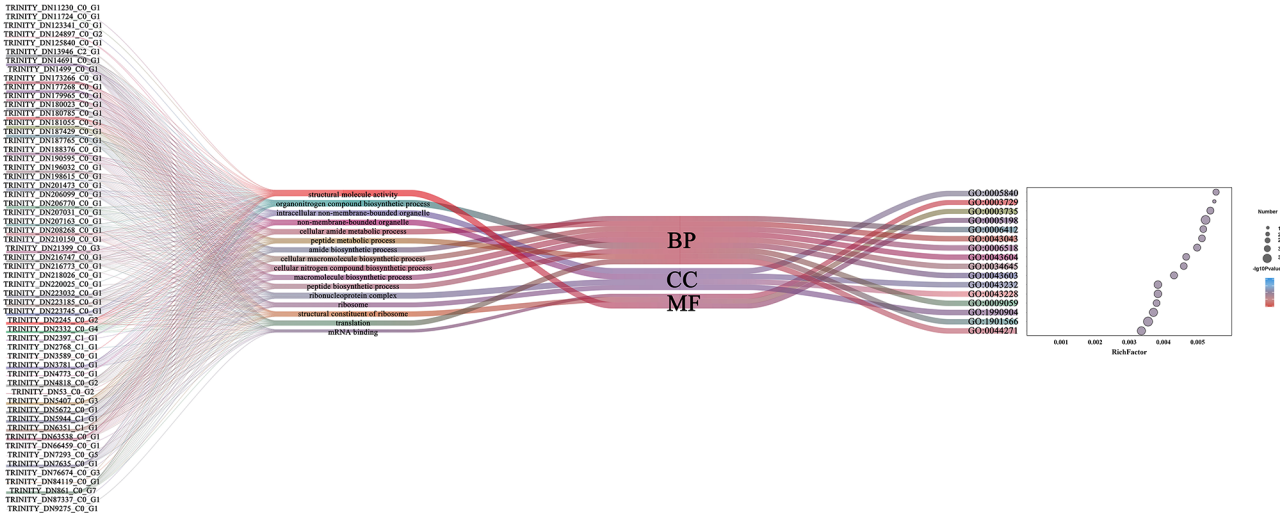


Fig. 4 Functional classification of DEGs and GO analysis. A: GO enrichment analysis of DEGs in the highlighted cluster based on the GO database. Z-score was assigned to x-axis and-log P value was assigned to the y-axis. The circles different in colors and size was positively proportional to the number of genes in a certain GO term. The GO above the threshold line indicate the largest number of genes and the lowest P-value. B: Sankey diagram representing the contributions of the DEGs enrichment. The four columns (from left to right) were DEGs, pathway, GO functional annotation, GO id. The bubble chart (on the rightmost) of GO enrichment shows the significant GO pathway, with -log P value on the x-axis and the number of genes that were enriched on the y-axis.

for validation via qRT−PCR. The expression levels of the selected genes were calculated via the $2^{-\triangle\triangle CT}$ method. A comparison of the expression data obtained by RNA-seq and qRT−PCR revealed that the expression trends of 16 genes were consistent with the transcriptome sequencing results. These findings indicate that the RNA-seq data are reliable and can be used for in-depth analysis (Fig. S6).

Discussion

The objective of this study was to investigate a novel salt-alkali-tolerant PGPR, *Zobellella* sp. DQSA1, which has the capacity to survive stably under salt-alkali stress conditions. This resulted in the promotion of the developmental growth and salt tolerance of Job's tears seedling. In the context of salt stress, the cocultivation of *Zobellella* sp. DQSA1 with Job's tears seedlings has been shown to alleviate the negative effects of salt stress on plant growth and development, which can be reflected indicators of.

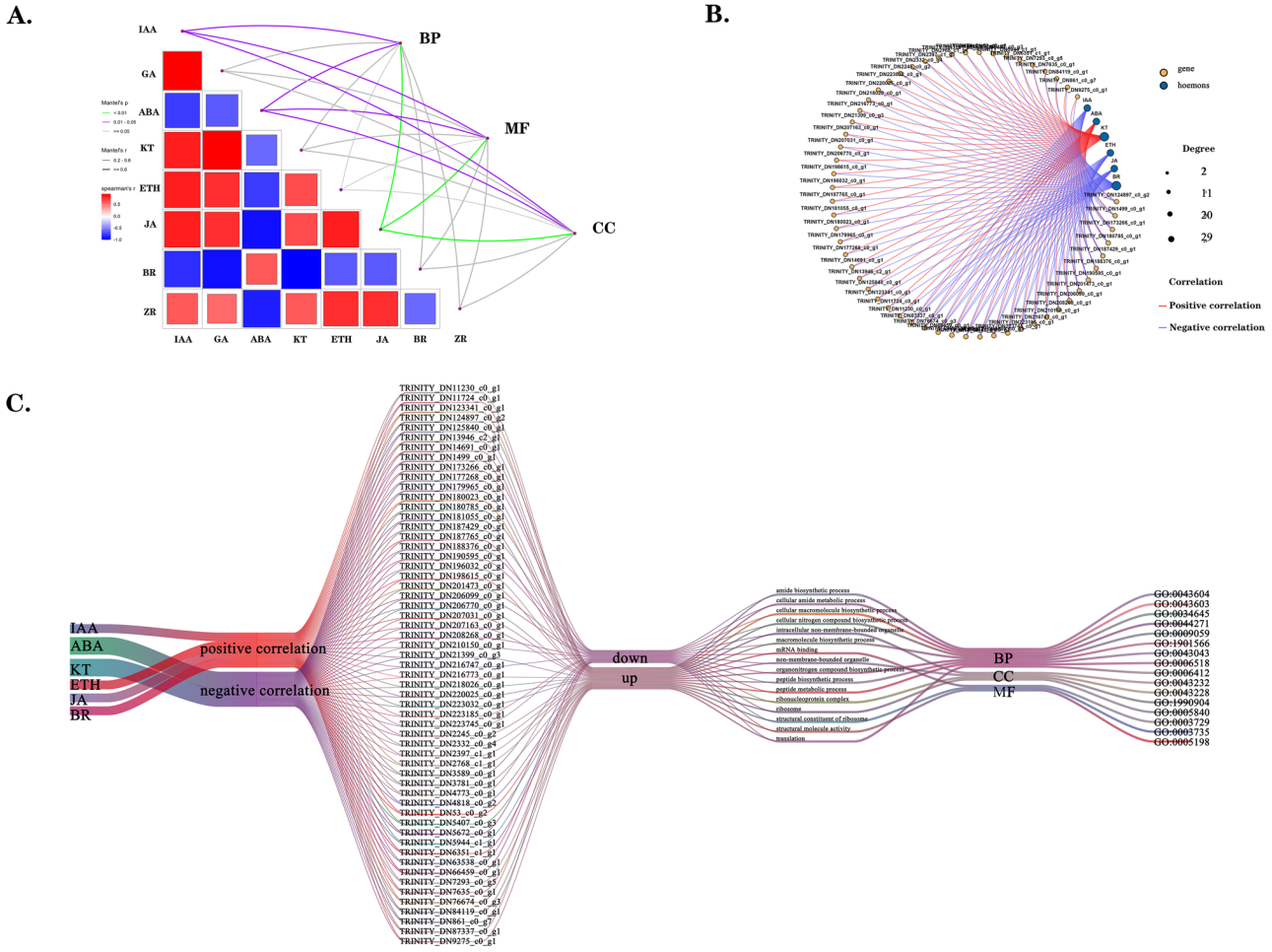


Fig. 5 Correlation analysis of key DEGs with endogenous hormone content. **A**: Correlation of key DEGs, GO, and endogenous hormone content. Mantel's analysis indicates that the functional composition of DEGs correlates with endogenous hormone content. Curve width is proportional to the Mantel's r statistic corresponding to the distance correlation, curve color indicates statistical significance based on Spearman's p-value, and color gradient indicates Spearman's correlation coefficient. **B**: Correlation analysis of endogenous hormone content with DEGs and GOs. Red lines indicate up-regulation and blue lines indicate down-regulation. **C**: Sankey diagram illustrating the contributions of endogenous hormone content to DEGs and functional genes. The seven columns (from left to right) were endogenous hormone content, correlation, DEGs, regulation, terms, catalog and GO, the value of Spearman's P-value < 0.05

root architecture, biomass accumulation and plant growth; the stimulation of phytohormone biosynthesis and signaling; and antioxidant capacity in experiment. These mechanisms act in a synergistic manner to promote the growth of Job's tears seedlings under salt-alkali stress. Plants have evolved symbiotic interactions with microbes that can alter plant phenotypic plasticity during plant growth or in response to stress [46]. By revealing the intricate regulatory mechanisms underlying plant–microbe interactions, we can gain insights into how PGPR can beneficially affect plant growth and stress tolerance. These findings have great potential for advancing biofertilizer development and sustainable agricultural practices [39].

Zobellella Sp. DQSA1 enhances the growth and salt tolerance of job's tear seedlings

Zobellella sp. DQSA1 can produce many amino acids through metabolism (Table S1), it was observed that the soluble protein content of SA + group was higher than that of SA group (Fig. 2), and the increase of soluble protein content may enhance the resistance of plants. Some amino acids have been clarified to enhance the antioxidant capacity of plant cells [52]. Considering that the nutrient solution used contained only *Zobellella* sp. DQSA1 and inorganic components, it is assumed that the observed increase in soluble protein content and subsequent enhancement of plant resistance may be attributed to the amino acids secreted by *Zobellella* sp. DQSA1. The study has shown that amino acids can be directly absorbed by plant roots [29] and potentially regulate plant resistance mechanisms.

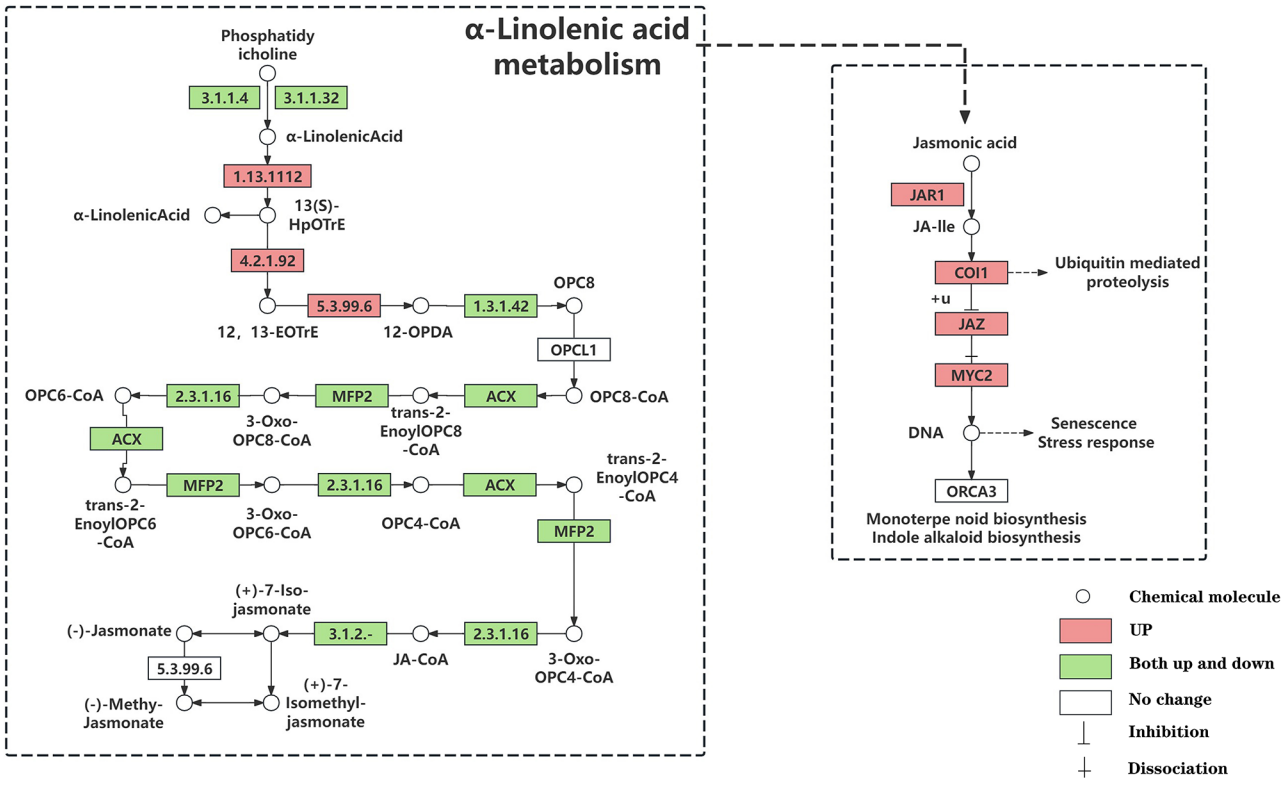


Fig. 6 KEGG analysis of JA-related pathways in Job's tears seedlings under salt-alkali conditions

Salt–alkali stress can lead to a range of changes in plants. In naturally salt-alkali soils, the principal salts present are sodium carbonate and sodium bicarbonate, which can cause synergistic damage by inducing high Na^+ toxicity, osmotic stress, and elevated pH stress in plants (Cheng [12]). When plants are subjected to salt stress, their growth and development are inhibited, resulting in stunted growth, wilting, or even death. This ultimately leads to a reduction in biomass [21]. In this study, the growth of Job's tears seedlings was initially evaluated under various salt–alkali conditions. These strains exhibited wilting but did not exhibit signs of mortality at a salt-alkali concentration of 30 mM (Fig. S2).

A number of studies have indicated that PGPRs such as *Bacillus*, *Enterobacter*, *Pseudomonas* and *Serratia* have the potential to alleviate the negative effects of salt-alkali stress and increase seed germination, chlorophyll synthesis and biomass production, thereby improving the growth and salt-alkali tolerance of certain crops [4, 24]. The data presented demonstrate that *Zobellella* sp. DQSA1 plays a pivotal role in enhancing the growth and salt tolerance of Job's tears seedlings (Fig. 1A). First, *Zobellella* sp. DQSA1 treatment promoted the growth of Job's tears seedlings, resulting in a significant increase in their dry and fresh weights (Fig. 1B-I). An examination of the root system revealed that although the root length was reduced, both the biomass and the number of root hairs in the root system increased significantly (Fig. 1J-M). Notably, the growth-promoting effect of *Zobellella* sp. DQSA1 was even more pronounced under salt-alkali conditions. The rationale for this is that this strain was discovered and screened in salt–alkali environments, which exhibit faster reproduction and increased activity under salt–alkali conditions [69].

Plants possess remarkable resilience, exemplified by their capacity to produce antioxidant enzymes that neutralize reactive oxygen species [3]. POD and CAT play pivotal roles in detoxifying H_2O_2 from seedling tissues and mitigating oxidative damage [23]. SOD reduces lipid peroxidation in cell membranes by inhibiting the production of hydroxyl radicals, thereby acting as the primary defense against reactive oxygen species [28]. Soluble substances serve to mitigate the effects of salt–alkali stress and maintain the intracellular ionic balance [11]. The present study also revealed that salt-alkali stress caused significant accumulation of H_2O_2, O^{2-}, and MDA in the leaves and roots of Job's tears seedlings. However, inoculation with *Zobellella* sp. DQSA1 significantly reduced the accumulation of H_2O_2, O^{2-}, and MDA (Fig. 2M-R) and increased the activities of antioxidant enzymes, such as CAT, POD, and SOD (Fig. 2A-F), as well as the contents of free proline, soluble proteins, and soluble sugars (Fig. 2G-L). These findings suggest that *Zobellella* sp. DQSA1 could improve the salt-alkali tolerance of Job's

tear seedlings by increasing the activities of antioxidant enzymes and osmotic regulators and eliminating toxic free radicals. These results are consistent with those of previous studies in which PGPR inoculation improved salt-alkali tolerance in different crops by enhancing the antioxidant defense system [32, 33, 67].

Zobellella Sp. DQSA1 enhances the salt-alkali tolerance of job's tear seedlings by regulating endogenous hormones in their root system

Endogenous hormones are signaling substances with complex and diverse physiological mechanisms. In response to stress, plants regulate their own hormone levels [65]. Among the various hormones involved in plant development, growth hormone plays a pivotal and indispensable role. IAA, the primary active component of growth hormone, has been shown to stimulate the growth of existing roots, the formation of adventitious roots, and the branching of roots. Salt–alkali stress has been shown to result in a notable decline in IAA content [59]. ZR and KT belong to a group of cytokinins that are involved in a range of physiological and biochemical processes in plants. These processes include cell division, the stem−root ratio, fertility, leaf senescence, and adaptation to abiotic stresses [37]. Cytokinins also inhibit root elongation and lateral root formation [50]. ABA plays a pivotal role in salt stress and is highly susceptible to stress. The accumulation of ABA closes the stomata to reduce the transpiration rate and initiates a complex network of signaling molecules in the plant to alleviate the ionic toxicity and oxidative damage caused by salt stress, especially osmotic stress. This process reduces or prevents the negative effects of salt stress on plant growth and ABA plays a protective role. ABA exerts a regulatory effect on root architecture, including the initiation and elongation of primary roots, lateral roots, adventitious roots and root hairs under both normal and stress conditions [61]. ABA acts as a regulatory center for plant hormones in response to stress and influences changes in the contents of other plant hormones. ABA inhibits lateral root growth and development by inducing ROS production in roots, antagonizing growth hormones and synergizing with cytokinins [35]. BR and JA are the most recently discovered hormones. BR plays a pivotal role in the regulation of plant root growth, development, and symbiosis. Furthermore, BRs regulate the size of root meristematic tissues and lateral root development in *Arabidopsis thaliana* and other root meristems over a range of concentrations [68]. JA is an essential endogenous signal that regulates a multitude of plant processes for environmental adaptation, which includes primary root growth inhibition and root hair elongation. JA affects root development by triggering the degradation of *JAZ* (jasmonate ZIM domain) deterrents and inducing transcriptional reprogramming through specific signaling pathways, such as the *COI1-JAZ* coreceptor pathway [34]. As a stress-related hormone, JA has also been reported to be involved in salt-induced growth inhibition. JA levels are elevated under salt stress and JA signaling is activated [63]. There is a paucity of research investigating the impact of gibberellin and ethylene on the root system. However, recent findings indicate that GA negatively regulates the radial growth of roots [58], some studies have shown that the change in GA concentration in plants under salt-alkali stress and drought stress significantly decreases and that plants can adapt to the external environment through a reduction in GA content [18]. Whereas ethylene plays a pivotal role in the development of the root system, including the formation of lateral roots and adventitious roots, the establishment of the microenvironment of the root stem cells and the development of root hairs [40], ethylene plays a complex role in the response of plants to saline-alkali stress. Although some studies have reported the positive regulatory role of ethylene in enhancing Arabidopsis tolerance (Dubois et al., 2018), others indicate that high levels of ethylene can adversely affect plant growth under stress conditions. Ethylene often mediates various adaptive responses that enhance tolerance to salinity stress [38, 47], highlighting its dual role depending on the environmental conditions and plant developmental stage.

In conclusion, the findings indicated that an increase in the contents of IAA, ABA, BR, and JA was associated with growth of lateral roots and root hairs. Conversely, an increase in the contents of ABA, JA, and CK was linked to a reduction in the elongation of primary roots. *Zobellella* sp. DQSA1 had a significant regulatory effect on the endogenous hormone levels of Job 's tears seedlings. Under saline-alkali stress, seedlings without inoculation of *Zobellella* sp. DQSA1 showed increased stress-related hormones such as ABA, JA, ETH and BR, while hormones that promote growth such as IAA, GA ZR and KT were significantly reduced, which indicates that plants preferentially activate stress response mechanisms under saline-alkali stress and sacrifices some growth ability to enhance viability. However, after inoculation with *Zobellella* sp. DQSA1, the levels of growth-promoting hormones such as IAA, GA, ZA and KT were significantly restored, while stress hormones such as ABA and BR were reduced accordingly. Consistent with other PGPR studies, *Zobellella* sp. DQSA1 can alleviate the growth inhibition caused by stress and help plants maintain growth in saline-alkali environment by regulating endogenous hormone levels. This hormone rebalancing mechanism may be achieved by directly or indirectly regulating the expression of key hormone synthases by *Zobellella* sp. DQSA1. Especially, the significant up-regulation of

JA signaling pathway-related genes further supports its important role in plant stress adaptation (Fig. 3).

Transcriptome and correlation analysis

The gene expression data suggest that Job's tear seedlings have a highly adaptive regulatory network of adapting to changing environmental conditions, as has been reported previously in wheat and rice [49]. One of the most noteworthy outcomes was the identification of a total of 173 key DEGs in Job's tear seedlings from different treatment groups (Table S2). These genes are likely candidates for further research on the genes that play key roles in promoting the growth and salt-alkali tolerance of Job's tear seedlings under the action of *Zobellella* sp. DQSA1. Among these key DEGs, both up-regulated and down-regulated genes were identified, underscoring the complexity and multifaceted nature of the regulatory mechanisms [43]. In addition, correlation analysis between the DEGs and hormone levels revealed the potential functional importance of these genes in regulating basic physiological responses to salt-alkali stress (Fig. 5), which is similar to previous research [62]. SA_vs_SA + has more DEGs than CK_vs_CK+, and the difference in the number of DEGs suggested that *Zobellella* sp. DQSA1 may have a more complex and variable molecular response mechanism under salt stress conditions (Fig. S5).

JA synthesis essentially involves a series of enzymatic reactions in which linolenic acid is released from the cell membrane as a substrate. Studies have shown that α-linolenic acid is catalyzed by lipoxygenase (*LOX*) to synthesize 13 S-hydroperoxylinolenic acid (13-HPOT) and 13-HPOT is catalyzed by allene oxide synthase (*AOS*) and allene oxide cyclase (AOC). Then, 13-HPOT is converted to 12-oxo-phytodienoic acid (*OPAD*) by *AOS* and *AOC*, which *OPAD* is translocated to peroxisomes, where it is converted to *OPDA* by oxidoreductase 3 (OPR3) and after three β-oxidations are converted to (+)-7-Iso-JA [36, 73]. Subsequently, (+)-7-Iso-JA is transported to the cytoplasm, where it binds to isoleucine (l-isoleucine, Ile) catalyzed by the ATP-dependent adenylate-forming enzyme (*JAR1*) to form the most biologically active jasmonate analogs recognized as (+)-7-Iso-JA-Ile [70], which culminates in the formation of biologically active JA-Ile. It has also been reported that *OPDA* can also synthesize 4,5-didehydrojasmonic acid (4,5-didehydro-JA,4,5-ddh-JA) in peroxisomes after three β-oxidations followed by OPR2-catalyzed synthesis of JA [14, 17]. When plants are in an ideal living environment, their endogenous JA content is very low and they lack biological activity. When plants are under stress, it is unable to respond directly to stress and regulate the growth of the plant due to the lack of JA activity. At this time, JA released into the cytoplasm combines with ATP and Ile through the *JAR1* protein to synthesize the most biologically active JA-like substance (JA-Ile), which is the most biologically active JA-like substance and rapidly are accumulated in plant cells (Fonseca et al., 2009a; Fonseca et al., [31]. Then, JA-Ile crosses the plasma membrane to the nucleus via *JAT1* transport and is recognized for function by the JA receptor *COI1* protein in the *SCF-COI*1 complex (consisting of an F-box protein, *COI1*, *SKP*, *CUL1*, and *RBX1*, among other proteins). The *COI1-JAZ* complex facilitates the interaction between *JAZ* and *COI1* by co-receptorizing JA-Ile and this interaction leads to the degradation of *JAZ* in the 26 S proteasome, which activates the transcription and expression of JA-responsive genes in TFs [5]. In this study, genes involved in the JA production and action pathways were found to be significantly up-regulated and the correlation with DEGs was the most significant, which suggests that *Zobellella* sp. DQSA1 may promote plant growth by regulating the level of JA (Figs. 5A and 6).

The transcriptome analysis performed provides important insights into the basis of salt-alkali tolerance in Job's tear seedlings in this study. In conclusion, the integration of functional classification, GO analysis and related tests facilitated a deeper understanding of the growth-promoting mechanism of *Zobellella* sp. DQSA1 in Job's tear seedlings under salt-alkali conditions.

Conclusion

Our dataset included whole-genome sequence analysis of *Zobellella* sp. DQSA1 and on the basis of the growth and physiological parameters of Job's tears seedlings being investigated, DEG profiling data that provide a dynamic perspective on transcriptomic variations in Job's tears seedling roots were comprehensively analyzed in response to *Zobellella* sp. DQSA1 under salt-alkali stress, which 173 DEGs with the greatest differences were identified. A Mantel test and co-occurrence network analysis were conducted to correlate the 57 DEGs with endogenous hormone contents and the correlation between JA and the 57 DEGs was found to be the most significant. The sequencing analysis indicated that *Zobellella* sp. DQSA1 can promote growth and salt–alkali stress tolerance by regulating endogenous hormone contents. These results not only have potential application value for the cultivation and production of Job's tears seedlings, but also provide a theoretical basis for further exploration of the function and mechanism of action of PGPR under salt-alkali stress.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06367-3.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Acknowledgements
Not applicable.

Author contributions
Y. L. and Y. H. conceived the research. Y. L., H. D., Y. H., D. X., S. Z., M. M. and T. Y. performed experiments. Y. L. and Y. H. analyzed the data. D. X., S. Z. and M. M. collected the plant materials, helped in the experiment and made suggestions. Y. L. and Y. H. wrote the manuscript. All authors have read and approved the manuscript.

Funding
This work was supported by the Natural Science Foundation of Heilongjiang Province (Grant number LH2023C077) and the Heilongjiang Bayi Agricultural University Postgraduate Innovative Research Project (Grant number YJSCX2023–Y67).

Data availability
The DNA data were deposited into the NCBI database under accession number PRJNA1156831. The RNA data were deposited into the NCBI database under accession number PRJNA1157917 and are available at the following URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1157917.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 21 August 2024 / Accepted: 7 March 2025
Published online: 20 March 2025

References

1. Afzal M, Nawaz K, Hussain K, Iqbal I, Riaz H, Rouf ZA, Javeria M, Akram R, Khaliq M, Attique A. Foliar application of mannitol as an effective approach to ameliorate the salt stress-induced toxicity in two varieties of maize (Zea Mays L). Pakistan Journal of Botany; 2022.
2. Aguilar L, Perez LM, Gallegos A. Effect of aeration on nitrogen removal-associated microbial community in an innovative vertical cork-based constructed wetland for winery wastewater treatment. Ecological engineering: The Journal of Ecotechnology; 2022.
3. Aguilera A, Tereucán G, Ercoli S, Cornejo P, Gomez MR, Uhlmann L, Guigas C, Esatbeyoglu T, Ruiz A. Influence of organic and chemical fertilisation on antioxidant compounds profiles and activities in fruits of Fragaria Ananassa Var. Camarosa. J Soil Sci Plant Nutr. 2020;20:715–24.
4. Akbar A, Han B, Khan A, Feng CX, Ullah A, Khan AS, He L, Yang X. A transcriptomic study reveals salt stress alleviation in cotton plants upon salt tolerant PGPR inoculation. Environmental and Experimental Botany; 2022.
5. Ali MS, Baek KH. Jasmonic acid signaling pathway in response to abiotic stresses in plants. Int J Mol Sci. 2020;21:621.
6. Armstrong J, Fiddes IT, Diekhans M, Paten B. Whole-genome alignment and comparative annotation. Annu Rev Anim Biosci. 2019;7(1).
7. Bates LS, Waldren R, Teare I. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39:205–7.
8. Cakmak I, Horst, Walter J. Effect of aluminum on lipid peroxidation, superoxide dismutase catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiologia Plantarum; 1991.
9. Cao Y, Zhang M, Liang X, Li F, Jiang C. Natural variation of an EF-hand Ca2+-binding-protein coding gene confers saline-alkaline tolerance in maize. Nat Commun. 2020;11:186.
10. Chen F, Huang Y, Cao Z, Li Y, Liu D, Han X, Yin K, Liu Q. New insights into the molecular mechanism of low-temperature stratification on dormancy release and germination of Saposhnikovia divaricata seeds. Brazilian J Bot. 2022;45:1183–98.
11. Chen L, Liu Y, Wu G, Veronican Njeri K, Shen Q, Zhang N, Zhang R. Induced maize salt tolerance by rhizosphere inoculation of Bacillus amyloliquefaciens SQR9. Physiologia Plantarum; 2016.
12. Cheng XZ, H.Banuelos G. Saline-alkaline tolerance of hygrophilous plant species during their asexual propagation and continued growth stages. S Afr J Bot. 2018;118:129–37.
13. Chialva M, Zhou Y, Spadaro D, Bonfante P. (2018). Not only priming: soil microbiota May protect tomato from root pathogens. Plant Signal Behav 13, e1464855.
14. Chini A, Monte I, Zamarre?O AM, Hamberg M, Lassueur S, Reymond P, Weiss S, Stintzi A, Schaller A, Porzel A. An OPR3-independent pathway uses 4,5-didehydrojasmonate for jasmonate synthesis. Nature Chemical Biology; 2018.
15. Chu TN, Tran BTH, Bui LV, Hoang MTT. (2019). Plant growth-promoting rhizobacterium Pseudomonas PS01 induces salt tolerance in Arabidopsis thaliana. BMC Research Notes *12*.
16. Chun-Li LI, Yuan-Yue S. Relationship between ABA and ethylene content and fruit ripening during Fig fruit development. Journal of China Agricultural University; 2016.
17. Claus W, Miroslav S. Jasmonates: news on occurrence, biosynthesis, metabolism and action of an ancient group of signaling compounds. Int J Mol Ences. 2018;19:2539.
18. Colebrook EH, Thomas SG, Phillips AL, Hedden P. The role of Gibberellin signalling in plant responses to abiotic stress. J Exp Biol. 2014;217:67–75.
19. de Vries FT, Griffiths RI, Knight CG, Nicolitch O, Williams A. Harnessing rhizosphere microbiomes for drought-resilient crop production. Science. 2020;368:270–4.
20. Debona D, Rodrigues FÁ, Rios JA, Nascimento KJT. (2012). Biochemical changes in the leaves of wheat plants infected by Pyricularia oryzae. Phytopathology *102*.
21. Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI. (2013). Plant salt-tolerance mechanisms.
22. Deng S, Ying Z, Yang Y, Lin Z, Chen M. Research progress of functional ingredients on adlay. Chin Agri Sci Bullet. 2017;33:123–8.
23. Desoky E-SM, Saad AM, El-Saadony MT, Merwad A-RM, Rady MM. Plant growth-promoting rhizobacteria: potential improvement in antioxidant defense system and suppression of oxidative stress for alleviating salinity stress in triticum aestivum (L.) plants. Biocatal Agric Biotechnol. 2020;30:101878.
24. Dong H, Wang Y, Di Y, Qiu Y, Ji Z, Zhou T, Shen S, Du N, Zhang T, Dong X. Plant growth-promoting rhizobacteria Pseudomonas aeruginosa HG28-5 improves salt tolerance by regulating Na+/K + homeostasis and ABA signaling pathway in tomato. Microbiol Res. 2024;283:127707.
25. Dubois, Van, den, Broeck. and Inze (2018). The Pivotal Role of Ethylene in Plant Growth. TRENDS PLANT SCI *2018,23(4)*, 311–323.
26. Earl HJ, Tollenaar M. Maize leaf absorptance of photosynthetically active radiation and its Estimation using a chlorophyll meter. Volume 37. Crop Science; 1997.
27. Egamberdieva D, Wirth SJ, Alqarawi AA, Abd_Allah EF, Hashem A. Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Front Microbiol. 2017;8:2104.
28. El-Esawi MA, Al-Ghamdi AA, Ali HM, Alayafi AA. Azospirillum lipoferum FK1 confers improved salt tolerance in Chickpea (Cicer arietinum L.) by modulating osmolytes, antioxidant machinery and stress-related genes expression. Environmental and Experimental Botany; 2018.
29. Fang-Fang X, Ming-Fu G, Chuan-Zhi Z, Chun-Ying XU, Ling-Chao F. Advances in the relationship between amino acid and plant stress resistance. Heilongjiang Agricultural Sciences; 2018.

30. Fonseca S, Chini A, Hamberg M, et al. (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. Nat Chem Biol. 2009;5(5):344–50.
31. Fonseca S, Chico JM, Solano R. The jasmonate pathway: the ligand, the receptor and the core signalling module. Curr Opin Plant Biol. 2009b;12:539–47.
32. Giannelli G, Mattarozzi M, Gentili S, et al. A novel PGPR strain homologous to Beijerinckia fluminensis induces biochemical and molecular changes involved in Arabidopsis thaliana salt tolerance. Plant Physiol Biochem. 2024;206:108187.
33. Giannelli G, Potestio S, Visioli G. The contribution of PGPR in salt stress tolerance in crops: unravelling the molecular mechanisms of cross-talk between plant and bacteria. Plants. 2023;12:2197.
34. Han X, Kui M, He K, Yang M, Du J, Jiang Y, Hu Y. Jasmonate-regulated root growth Inhibition and root hair elongation. J Exp Bot. 2023;74:1176–85.
35. Huan D, Ling B, Chunpeng S. Growth and development of lateral root regulated by plant hormone ABA. Chinese Agricultural Science Bulletin; 2016.
36. Huang H, Bei L, Liangyu L, et al. Jasmonate action in plant growth and development. J Exp Bot. 2017;(6):1349–59.
37. Hyoung S, Cho SH, Chung JH, So WM, Cui MH, Shin JS. Cytokinin oxidase PpCKX1 plays regulatory roles in development and enhances dehydration and salt tolerance in physcomitrella patens. Plant Cell Rep. 2020;39:419–30.
38. Khan S, Alvi AF, Khan NA. Role of ethylene in the regulation of plant developmental processes. Volume 4. Stresses; 2024. pp. 28–53.
39. Kumawat KC, Sharma B, Nagpal S, Kumar A, Tiwari S, Nair RM. (2023). Plant growth-promoting rhizobacteria: Salt stress alleviators to improve crop productivity for sustainable agriculture development. Frontiers in plant science *13*, 1101862.
40. Jintao L, Yuna Y, Mengmeng C, et al. Research of ethylene modulated auxin biosynthesis and transport in rice root. J Xinyang Normal Uni (Nat Sci Ed). 2020;33(3):377–84.
41. Liu X, Jin Y, Tan K, Zheng J, Gao T, Zhang Z, Zhao Y, Ma F, Li C. MdTyDc overexpression improves alkalinity tolerance in Malus domestica. Front Plant Sci. 2021;12:625890.
42. Livak K.J., Schmittgen T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 2001;25:402–8.
43. Lv J, Liu P, Wang Y, Gao B, Chen P, Li J. Transcriptome analysis of portunus trituberculatus in response to salinity stress provides insights into the molecular basis of osmoregulation. PLoS ONE. 2013;8:e82155.
44. Lv P, Luo J, Zhuang X, Zhang D, Huang Z, Bai Z. Diversity of culturable aerobic denitrifying bacteria in the sediment, water and biofilms in Liangshui river of Beijing, China. Sci Rep. 2017;7:10032.
45. Maia J, Costa de Macedo C, Voigt E, Freitas J, Silveira J. Antioxidative enzymatic protection in leaves of two contrasting Cowpea cultivars under salinity. Biol Plant. 2010;54:159–63.
46. Mellidou I, Ainalidou A, Papadopoulou A, et al. Comparative transcriptomics and metabolomics reveal an intricate priming mechanism involved in PGPR-mediated salt tolerance in tomato. Front Plant Sci. 2021;12:713984.
47. Qu X, Hall BP, Gao Z, Schaller GE. A strong constitutive ethylene-response phenotype conferred on Arabidopsis plants containing null mutations in the ethylene receptors ETR1 and ERS1. BMC Plant Biol. 2007;7:3.
48. Ren H, Zhang B, Zhang F, Liu X, Wang X, Zhang C, Zhao K, Yuan R, Lamlom SF, Abdelghany AM. Integration of physiological and transcriptomic approaches in investigating salt-alkali stress resilience in soybean. Plant Stress. 2024;11:100375.
49. Ren X, Shan Y, Li X, Fan J, Li Y, Ma L, Wang L, Li X. Application of RNA sequencing to understand the benefits of endophytes in the salt-alkaline resistance of rice seedlings. Environmental and experimental botany; 2022. p. 196.
50. Rivas MÁ, Friero I, Alarcón MV, Salguero J. Auxin-cytokinin balance shapes maize root architecture by controlling primary root elongation and lateral root development. Front Plant Sci. 2022;13:836592.
51. Rodrigues FCT, Silveira PR, Cacique IS, Oliveira LM, Rodrigues FA. Soybean plants sprayed with Azelaic and hexanoic acids and infected by phakopsora pachyrhizi display better photosynthetic performance and a robust antioxidative metabolism. J Phytopathol. 2023;171:320–32.
52. Romanenko KO, Babenko L, Kosakivska I. Amino acids in regulation of abiotic stress tolerance in cereal crops: a review. Cereal Research Communications; 2023.
53. Rosinger CH, Wilson JM, Kerr MW. Changes in the soluble protein and free amino acid content of chill-sensitive and chill-resistant plants during chilling and hardening treatments. J Exp Bot. 1984;(10): 1460–71.
54. Safdarian M, Askari H, Shariati JV, et al. Transcriptional responses of wheat roots inoculated with Arthrobacter nitroguajacolicus to salt stress. Sci Rep. 2019;9(1):1792.
55. Kumar S, Agarwal S, Ranvijay. Fast and memory efficient approach for mapping NGS reads to a reference genome. J Bioinform Comput Biol. 2019;17(02):1950008.
56. Shams EA, Guleray. Salinity induced alteration in DNA methylation pattern, enzyme activity, nutrient uptake and H2O2 content in pepper (Capsicum annuum L.) cultivars. Volume 42. Acta Physiologiae Plantarum; 2020.
57. Shavalikohshori O, Zalaghi R, Sorkheh K, Enaytizamir N. The expression of proline production/degradation genes under salinity and cadmium stresses in triticum aestivum inoculated with Pseudomonas Sp. Int J Environ Sci Technol. 2020;17:2233–42.
58. Shi-hui N, Wei L, Xiao-yang C. Negative regulation of Gibberellin on root tip diameter. J Beijing Forestry Univ. 2013;35:77–83.
59. Singh AK. In vitro studies of salinity stress response in allium sativum. Allied Academies; 2021.
60. Song Y, Cui X, Chen J, Yang R, Yan X. The profiling of eleven phytohormones in under different high-temperature environments. Journal of Fisheries of China; 2017.
61. Teng Z, Lyu J, Chen Y, Zhang J, Ye N. Effects of stress-induced ABA on root architecture development: positive and negative actions. Crop J. 2023;11:1072–9.
62. Thoppurathu FJ, Ghorbanzadeh Z, Vala AK, Hamid R, Joshi M. Unravelling the treasure trove of drought-responsive genes in wild-type peanut through transcriptomics and physiological analyses of root. Funct Integr Genom. 2022;22:215–33.
63. Valenzuela CE, Acevedo-Acevedo O, Miranda GS, Vergara-Barros P, Holuigue L, Figueroa CR, Figueroa PM. Salt stress response triggers activation of the jasmonate signaling pathway leading to Inhibition of cell elongation in Arabidopsis primary root. J Exp Bot. 2016;67:4209–20.
64. Venkatasubbaiah P, Krishna Y, Kaledhonkar M. Soil properties, land use and livelihood options in salt-affected areas of YSR Kadapa district. Andhra Pradesh, India: Current Science; 2022.
65. Waadt R, Seller CA, Hsu P-K, Takahashi Y, Munemasa S, Schroeder JI. Plant hormone regulation of abiotic stress responses. Nat Rev Mol Cell Biol. 2022;23:680–94.
66. Wang C, Wang H, Li Y, Li Q, Yan W, Zhang Y, Wu Z, Zhou Q. Plant growth-promoting rhizobacteria isolation from rhizosphere of submerged macrophytes and their growth-promoting effect on vallisneria natans under high sediment organic matter load. Microb Biotechnol. 2021a;14:726–36.
67. Wang G, Zhang L, Zhang S, Li B, Li J, Wang X, Zhang J, Guan C, Ji J. The combined use of a plant growth promoting Bacillus Sp. strain and GABA promotes the growth of rice under salt stress by regulating antioxidant enzyme system, enhancing photosynthesis and improving soil enzyme activities. Microbiol Res. 2023;266:127225.
68. Wang L, Yang R, Sun J. Regulation of crop agronomic traits and abiotic stress responses by brassinosteroids: a review. Sheng Wu Gong Cheng Xue bao = chinese. J Biotechnol. 2022;38:34–49.
69. Wang Y, Xiang J, Zhou Y, Liu Q, Yin K, Zhang X. Isolation and identification of saline▪ alkali tolerance bacteria DQSA1 and its growth▪ promoting effect on mung bean under saline▪ alkali stress. Microbiol China. 2021b;48:2653–64.
70. Wasternack C. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Botany. 2007;100:681–97.
71. Weihuan J, Lan C, Yong L. Cadmium negatively affects the growth and physiological status and the alleviation effects by exogenous selenium in silage maize (Zea Mays L). Environ Sci Pollut Res. 2024;31:21646–58.
72. Xi X-J, Zhu Y-G, Tong Y-P, Yang X-L, Tang N-N, Ma S-M, Li S, Cheng Z. Assessment of the genetic diversity of different job's tears (Coix lacryma-jobi L.) accessions and the active composition and anticancer effect of its seed oil. PLoS ONE. 2016;11:e0153269.
73. Yang Z, Huang Y, Yang J, Yao S, Li Y. Jasmonate signaling enhances RNA Silencing and antiviral defense in rice. Volume 28. Cell Host & Microbe; 2020.
74. Zhang Y. New research progress of active ingredients in job's tears. Hans J Food Nutr Sci. 2015;04:87–91.

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