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Rhizosphere microorganisms mediate ion homeostasis in cucumber seedlings: a new strategy to improve plant salt tolerance

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Abstract

Background Soil salinization is a formidable challenge for vegetable production, primarily because of the detrimental effects of ion toxicity. Rhizosphere microorganisms promote plant growth and bolster salt tolerance, but the extent to which microbial communities can increase plant resilience by regulating ion homeostasis under salt stress remains underexplored. The goal of this study was to enrich microbial communities from the rhizosphere of salt-stressed cucumber seedlings and identify their impact on ion balance and plant growth under saline conditions.

Results Salt stress induced significant alterations in the composition, structure, and function of the root-associated microbial community. Compared with a 75 mM NaCl treatment alone, inoculation with salt-induced rhizosphere microorganisms (SiRMs) under the same conditions significantly increased the growth of cucumber seedlings; plant height increased by 61.3%, and the fresh weights of the shoots and roots increased by 45.3% and 38.9%, respectively. Moreover, superoxide dismutase (SOD) activity increased by 4.1%, and peroxidase (POD) activity and superoxide anion ($O_2 \cdot^-$) content decreased by 10.5% and 3.7%, respectively. In the roots, stems, and leaves of cucumber seedlings treated with SiRMs and 75 mM NaCl, the Na^+ content was significantly reduced by 15.8%, 18.9%, and 9.7%, respectively. Conversely, the K^+ content significantly increased by 32.7%, 16.9%, and 28.8%, respectively. Under salt stress conditions, inoculation with SiRMs significantly increased the rate of Na^+ expulsion in the roots of cucumber seedlings by 18.3%, but the K^+ expulsion rate decreased by 76.7%. These dynamic changes are attributed to the upregulation of genes such as *CsHKT1*, *CsHAK5*, and *CsCHX18;4*.

Conclusions Enrichment with SiRMs played a pivotal role in maintaining ion homeostasis and significantly enhanced the salt tolerance of cucumber seedlings. These findings highlight the potential for microbial-assisted strategies to mitigate the adverse effects of soil salinity and provide valuable insights into the complex interplay between the microbial community and plant resilience from the perspective of ion balance.

Keywords Cucumber, Salt stress, Rhizosphere microorganisms, Ion homeostasis

Introduction

Cucumber (*Cucumis sativus* L.) is a widely cultivated vegetable and is renowned for its high yield and significant economic returns [1]. However, cucumber plants have a relatively shallow root distribution in the soil. This physiological characteristic makes the plants exceptionally sensitive to changes in the soil environment, particularly salinity. Once a plant is subjected to salt stress, cucumber yield and quality can significantly decline [2,

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3]. In greenhouses, a lack of rainwater leaching coupled with the excessive application of water and fertilizer causes salt in the soil to migrate upwards and accumulate on the surface as water evaporates. The accumulation of salt in the soil causes the progressive deterioration of the plant growth environment [4, 5].

More than 1.381 billion hectares of land (accounting for 10.7% of global land) are affected by salinization [6]. High concentrations of salt ions in the soil can inhibit plant growth and disturb physiological metabolism. First, salt stress reduces soil water potential, thereby inhibiting root water uptake and impeding plant growth [7]. Second, salt stress induces the accumulation of reactive oxygen species (ROS) in plants, which may damage chloroplasts and cause oxidative stress, thus inhibiting photosynthesis [8]. Excessive salt ion accumulation also limits nutrient ion absorption and causes nutrient imbalances and deficiencies that negatively affect plant health [9]. Notably, excessive Na^+ accumulation disrupts osmotic potential and ion homeostasis in plants, which impairs growth, development, and metabolic processes. For example, when *Arabidopsis thaliana* is exposed to a high-salt environment, excessive accumulation of Na^+ within plant tissues results in cell necrosis and ultimately leads to plant death [10]. Similarly, in tomatoes, the accumulation of salt in the apoplast disrupts cellular ion homeostasis and causes plant dehydration and wilting [11]. When the soil contains high levels of salt, plants passively accumulate large amounts of Na^+, which leads to osmotic stress. Since the hydration radius of Na^+ is similar to that of K^+ and its ionic radius is comparable to that of Ca^{2+}, the absorption of K^+ and Ca^{2+} by salt-stressed plants is inhibited, and imbalances in the K^+/Na^+ and Ca^{2+}/Na^+ ratios in plants may occur [12, 13]. Additionally, Cl^- is transported to various parts of salt-stressed plants via transpiration. When the concentration of Cl^- reaches a critical threshold, it interferes with the uptake of other essential nutrients, causes nutrient imbalances, and may have toxic effects [14]. Overall, ion imbalance reduces or even eliminates cell-selective permeability, and excessive accumulation of Na^+ and Cl^- in cells leads to physiological dysfunction that inhibits plant growth [15, 16].

In recent years, the adaptability of rhizosphere microorganisms to saline–alkaline environments and the mechanisms by which they promote plant growth have become research foci in the field of soil ecology. Beneficial rhizosphere microorganisms, including plant growth-promoting rhizobacteria (PGPR) [17], arbuscular mycorrhizal fungi (AMF) [18], and rhizobia [19], colonize rhizosphere or plant tissues and promote growth, improve nutrient uptake, and confer tolerance to abiotic and biotic stresses. Salt-tolerant soil PGPR, such as *Arthrobacter*, *Azospirillum*, *Alcaligenes*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Pseudomonas*, and *Rhizobium* can be utilized as biological inoculants to increase soil organic matter, improve the soil structure, and increase the water-holding capacity of the soil, thereby mitigating salt stress [20, 21]. Some rhizobacteria can secrete exopolysaccharides (EPSs) in the rhizosphere to mitigate salt stress. The hydroxyl, carboxyl, and phosphoryl functional groups within EPSs can chelate Na^+ ions and block their entry into plant tissues in the rhizosphere microdomain, thus reducing ionic toxicity [22, 23]. AMF increase plant tolerance to salt stress by regulating the expression of specific plant genes [24]. For example, AMF promote Na^+ efflux by upregulating the expression of *OsSOS1* and *OsHKT2;1*, and the sequestration of Na^+ in root cell vacuoles through the upregulation of *OsNHX3* effectively reduces Na^+ transport to aerial parts and mitigates ionic toxicity [25–28]. In addition, AMF and PGPR synergistically alleviate ionic toxicity by modulating the expression of K^+ transporter (HKT), Na^+/H^+ antiporter (NHX), and SOS pathway genes. For example, *Bacillus amyloliquefaciens* SQR9 can regulate the expression of Na^+ efflux-related genes (*HKT1*, *NHX1*, *NHX2*, and *NHX3*) in maize and promote Na^+ excretion [29, 30]. Additionally, the inoculation of soybean plants with rhizobia under salt stress conditions can regulate the expression of *GwNHX1*, which enhances the plant response to salt stress. Rhizobia can increase the levels of NO_3^-, NH_4^+, and total nitrogen in plants while reducing Na^+ and Cl^-, thereby increasing plant salt tolerance [31].

The importance of microorganisms in plant resistance to salt stress has been previously confirmed, but research has focused on the promotion of plant salt tolerance by individual or a few microorganisms, neglecting the fact that microorganisms associated with plants in nature exist in highly complex biological communities within plants, on plant surfaces, and in rhizosphere and phyllosphere environments. The group and in situ effects of these microbial communities on plant salt tolerance are poorly understood. In this study, we investigated whether rhizosphere microorganisms could increase salt tolerance in cucumber plants from a microbial community perspective. Maintaining ion balance in plants is crucial for cultivating salt-tolerant crops. Specifically, we asked whether cucumber rhizosphere microorganisms regulated the ion balance in cucumber seedlings under salt stress. Addressing these questions improves our understanding of the mechanisms underlying microbial assistance in plant salt tolerance and provides new insights into improving salt tolerance in crop plants.

Materials and methods

Plant materials and treatments

The soil used in this study was collected from a pesticide- and chemical fertilizer-free plantation in Xinjiang, China (43°56′37″N, 87°21′21″E). The upper 20 cm layer of the soil was collected, sieved to ensure a particle size of less than 2 mm, and analysed for physicochemical properties. The soil had a pH of 7.16, electrical conductivity of 1478.66 $\mu s \cdot cm^{-1}$, organic matter content of 13.41 $g \cdot kg^{-1}$, total nitrogen content of 0.99 $g \cdot kg^{-1}$, available phosphorus content of 156.99 $mg \cdot kg^{-1}$, and available potassium content of 306.88 $mg \cdot kg^{-1}$. Cucumber seeds ('Xintai Mici', obtained commercially from Xinjiang Lianchuang Seed Industry Co., Ltd., China) were surface-sterilized with 70% ethanol for 30 s, rinsed three times with sterile water, surface-sterilized with 3% sodium hypochlorite solution for 10 min, and then rinsed three additional times with sterile water. The sterilized seeds were germinated in a constant-temperature incubator at 28 °C, and seeds were sown after germination. The plants were grown in a growth chamber with a mean temperature of 26 °C/18 °C, a light/dark cycle of 14/10/h, a relative humidity of 60–80%, a CO_2 concentration of 380 $\mu mol \cdot mol^{-1}$, and average daily photosynthetically active radiation of 278 ± 8.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the light period.

The microbial suspension treatment was initiated when first true leaf on the seedlings had fully expanded. The soil was watered with a salt-induced rhizosphere microbial (SiRM) suspension (OD_{600} = 1.0) to saturation every 3 days, with sterile water serving as the control. When the seedlings reached the two-leaf stage, salt treatment was initiated. Plants were irrigated every 3 days with sterile Hoagland–Arnon nutrient solution containing either 0 mM or 75 mM NaCl for a total duration of 15 days. To avoid salt shock, the NaCl concentration in the nutrient solution was gradually increased by 25 mM per day until a final concentration of 75 mM was reached. The experiment included four treatments: (i) Control: plants received sterile water; (ii) Rh: plants were inoculated with SiRMs under nonsaline conditions; (iii) Na: plants received 75 mM NaCl; and (iv) RN: plants were inoculated with SiRMs under 75 mM NaCl conditions.

Measurements of plant parameters associated with salt tolerance

To assess the inhibitory effects of salt stress on cucumber seedling growth, we measured morphological and physiological indicators. The morphological parameters included plant height, stem diameter, root length, root surface area, and root volume, and eight plants from each treatment were measured. The physiological indices included superoxide dismutase (SOD), peroxidase (POD), and superoxide anion ($O_2 \cdot^-$) contents, which were determined with kits (Beijing Boxbio Science & Technology Co., Ltd.), and four plants from each treatment were measured. Root activity was determined by using the TTC reduction method (each treatment was replicated 8 times, one of which was the control). Photosynthetic parameters, including the leaf net photosynthesis, stomatal conductance, intercellular CO_2 concentration, and transpiration, were measured with a Li-6400 photosynthesis system (LI-COR, USA). Each treatment was repeated 4 times, and each repeat was measured 4 times. The nutrient elements P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn, and Mo were determined, and the ratio of a nutrient element was calculated and visualized as a radar chart. Each treatment was repeated 3 times. Further details regarding these methods are provided in the Supplementary Materials and Methods.

Plant ion content and root ion flux

To determine the ion content of a plant, 0.1 g of dried and ground sample was weighed into a 50 mL centrifuge tube and 20 mL of 1 M HCl was added. The mixture was placed on a shaker at 100 rpm for 5 h. After filtration, 1 mL of the filtrate diluted fivefold with deionized water. Ion concentrations were measured with a flame photometer, and each treatment was repeated 3 times. H^+-ATPase activity was measured with a kit according to the manufacturer's instructions, and each treatment was repeated 6 times.

To investigate the effect of rhizosphere bacterial enrichment on ion uptake, we measured the steady-state ion flux (Na^+, K^+, H^+, and Cl^-) at the root tip with noninvasive microtest technology (NMT) provided by XUYUE (Beijing) Technology Co. Measurements were taken at a distance of 1500 μm from the root tip of plants. The ion flux rate data were recorded directly using imFluxes V2.0 software (Younger USA LLC, Amherst, MA, USA), and each treatment was repeated 3 times. Detailed methods are provided in the Supplementary Materials.

Sampling, isolation, and culture of microorganisms

Bulk soil, rhizosphere, and root samples were collected following the protocol of Niu et al. (2017) [32]. Briefly, soil more than 3 cm away from the roots was designated bulk soil, while soil within approximately 1 mm of the roots was defined as the rhizosphere. Roots were carefully excised from the plants, and loose soil was shaken off, leaving a thin layer (approximately 1 mm) of adhering soil around the roots. To prevent contamination from the pot–soil interface, roots at this junction were not collected. The remaining rhizosphere soil was gently washed off using sterile 1× phosphate buffered saline solution (PBS, pH = 7.4). The buffer used for washing was centrifuged, and the resulting precipitate was retained as the

rhizosphere sample. The cleaned roots were sterilized and checked for contamination [33]. The root sections confirmed to have no live microbes or bacterial DNA on the surfaces were considered the root compartment and were used for the extraction of endospheric microbes. Samples were immediately frozen in liquid nitrogen and stored at −80 °C until DNA extraction. Further details are provided in the Supplementary Materials and Methods.

Additionally, the rhizosphere soil from cucumber seedlings in the salt treatment was homogenized with 1 × PBS buffer to prepare a 0.1 $g{\cdot}mL^{-1}$ suspension, and 1 mL of this suspension was inoculated into R2A liquid medium for enrichment and culture at 28 °C and 180 rpm on a rotary shaker for 48 h. When the concentration of the bacterial culture reached an OD600 of 1.0, it was used for subsequent inoculation verification [33].

DNA extraction, PCR amplification, and sequencing

Cucumber root endophytes, rhizosphere soil, and bulk soil microbial DNA were extracted using the PowerSoil DNA Isolation Kit according to the manufacturer's instructions. Before microbial DNA amplification and sequencing, the quality of the extracted DNA was assessed. After passing a quality control test, microbial DNA samples were amplified using the forward primer 799F (5'- AACMGGATTAGATACCCKG -3') and the reverse primer 1193R (5'- ACGTCATCCCCACCTTCC -3') to target the V5-V7 region of the bacterial 16S rRNA. The PCR procedure consisted of initial denaturation at 95 °C for 3 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. Sample libraries for sequencing were prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina, San Diego, CA, USA) as described previously [34] and subjected to a single sequencing run on a MiSeq PE300 platform (Illumina, Inc., San Diego, CA, USA).

The resulting sequences were quality filtered with fastp (0.19.6) and merged using FLASH (version 1.2.7). High-quality sequences were then denoised via the DADA2 plugin in QIIME2 (version 2022.2) [35], which yields single-nucleotide resolution on the basis of error profiles within samples. The denoised sequences obtained through DADA2 are referred to as amplicon sequence variants (ASVs). Taxonomic assignment of bacteria was performed using the classify-sklearn (Naive Bayes) consensus taxonomy classifier against the SILVA 138/16S_bacteria database [36].

RNA-seq library preparation, construction, and analysis

Cucumber root samples were collected for total RNA extraction using RNA Purification Reagent according to the manufacturer's instructions. RNA degradation and contamination were monitored on 1% agarose gels. RNA quantification was performed with a Nanodrop 2000 spectrophotometer. RNA purification, reverse transcription, library construction, and sequencing were conducted at Shanghai Majorbio Biopharm Biotechnology Co., Ltd. (Shanghai, China) following the manufacturer's protocols (Illumina, San Diego, CA). To identify differentially expressed genes (DEGs) between the two sample groups, the expression level of each gene was calculated using the fragments per kilobase of transcript per million mapped reads (FPKM) method. Differential expression analysis was performed using DESeq2, and DEGs with a $|\log_2(\text{fold change})| \geq 2$ and $P \leq 0.05$ were considered to be significantly differentially expressed genes.

Gene expression by qRT–PCR

Total RNA from cucumber roots was extracted with a column-type total plant RNA extraction and purification kit (Shanghai Sangon) according to the manufacturer's instructions. The concentration and OD260/280 of the RNA samples were assessed with a Nanodrop 2000 spectrophotometer, and RNA integrity was assessed by agarose gel electrophoresis. cDNA was synthesized by reverse transcription following the protocol provided with the kit (Shanghai Sangon).

Primers (Table S1) were designed with Primer 3 Plus online software (https://www.primer3plus.com), and 3–5 pairs of primers were designed as alternatives for each gene. Primer specificity was verified using NCBI BLAST. The *Actin* gene was used as the internal reference gene. Quantitative real-time PCR was performed using the SuperReal PreMix Plus (SYBR Green) Kit (Tiange Biotech, Beijing, China), and the relative expression levels of the genes were calculated according to the $2^{-\triangle\triangle CT}$ method [37].

Statistical analyses

Plant growth and physiological indices were analysed using analysis of variance (ANOVA) followed by Duncan's multiple range test for multiple comparisons with SPSS 23 software. The results were visualized in Origin 2021 and R (version 4.2.1). The experimental data are presented as the means ± standard deviations. Student's t tests were used for differences in microbial community composition. Microbiome data were analysed with a principal coordinate analysis (PcoA) at the ASV level on the basis of the Bray–Curtis distance in R. Group differences were tested using ANOSIM. To identify the potential functions of bacteria, we referred to the FAPROTAX database (http://www.zoology.ubc.ca/louca/FAPROTAX), which converts microbial community profiles into putative functional profiles on the basis of the current literature on cultivated strains. We focused on several

ecologically important functional groups related to plant growth, such as those related to photosynthesis and to carbon, nitrogen, and sulfur metabolism. Microbial function prediction was conducted at the ASV level using Kruskal–Wallis tests, which were corrected for multiple testing using the false discovery rate. Post hoc significance was assessed with a Tukey–Kramer test at the 0.05 level. A Circos diagram of the microbial community was generated with Circos-0.67–7 (http://circos.ca/) at the genus level, and species with relative abundances less than 0.01 were merged into an "others" category. Transcriptome data were analysed using DESeq2 for differential expression, with a screening threshold of $|\log_2 FC| >= 1$ and $P < 0.05$. Gene Ontology (GO) enrichment analysis of the gene set was performed with Goatools using Fisher's exact test. Enrichment was considered significant when $P < 0.05$, and the results were visualized using R.

Results

Bacterial community, structure, and functional responses to salt stress

High-throughput sequencing was conducted on rhizosphere soil (Rh), root endosphere (R), and bulk soil (S) samples from the control and salt stress treatments to investigate the specific bacterial community recruited by cucumber in response to salt stress. We constructed 16S rRNA amplicon libraries targeting the V5-V7 regions using the 799F and 1193R primers. After sequencing, we obtained a total of 24,886 ASVs (Supplementary Table S2). At the phylum level, the relative abundance of *Proteobacteria* in the rhizosphere soil increased significantly (by 1.33-fold) in the salt treatment compared with the control ($P < 0.05$), whereas the abundances of *Chloroflexi*, *Verrucomicrobiota*, *Acidobacteriota*, *Bdellovibrionota*, and *Planctomycetota* decreased by 0.62-, 0.32-, 0.39-, 0.38-, and 0.29-fold, respectively ($P < 0.05$) (Fig. 1a and Table S3). A PCoA revealed significant differences in microbial community composition between the control and salt stress treatments, particularly in the rhizosphere soil and root endosphere (Fig. 1b). A constrained principal coordinate analysis (CPCoA) indicated that salt treatment accounted for 24.6% of the microbial variation ($P < 0.001$), whereas different sampling methods explained 55.5% of the variation ($P < 0.001$) (Fig. 1c, d). A functional prediction of the microbial community revealed that functions related to nutrient cycling were significantly altered, including chemoheterotrophy, nitrate reduction, aromatic compound degradation, ureolysis, methylotrophy, methanol oxidation, and nitrogen fixation (Kruskal–Wallis rank sum test, $P < 0.05$; Fig. 1e and Table S4).

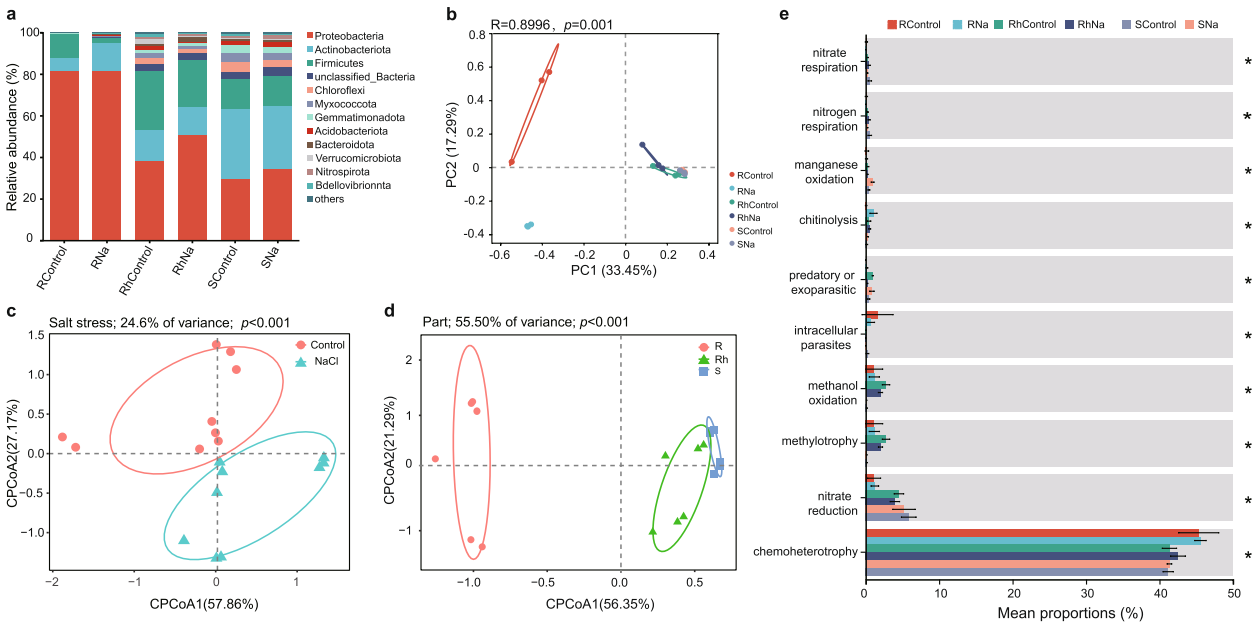

Fig. 1 Root-associated bacterial community composition, structure, and function under salt stress. **a** Relative abundance of bacteria at the phylum level; the "others" category includes phyla with a relative abundance less than 1%. **b** Principal coordinate analysis (PCoA) for the microbial community structure and **c** constrained principal coordinate analysis (CPCoA) for the microbial community structure in the salt stress treatment and **d** across sampling locations. **e** A functional prediction of microbial communities using FAPROTAX shows the top 10 functions based on summed mean values. A Kruskal–Wallis rank sum test was used for multiple group comparisons. The horizontal axis indicates the function name, the vertical axis shows the percentage abundance of each function within the sample, different colours represent different subgroups, and *P* values are indicated on the far right. An * indicates $0.01 < P \leq 0.05$

These findings demonstrate that salt stress induced significant changes in the structure, composition, and functional potential of the microbial community associated with cucumber roots.

Salt-induced rhizosphere microorganisms increase plant resistance to salt stress

We collected, cultured, and sequenced SiRMs (Fig. 2a), which were predominantly in the genus *Bacillus* (Fig. 2b). To evaluate the effects of SiRMs, we measured growth indices for seedlings in each treatment. Salt stress significantly inhibited the growth of the cucumber seedlings, but the addition of SiRMs significantly alleviated this inhibition. Compared with cucumber seedlings in the Na treatment, plant height, shoot fresh weight, and root fresh weight of seedlings in the RN treatment increased by 61.3%, 45.3%, and 38.9%, respectively (Fig. 2c–e). Stem diameter, shoot dry weight, and root dry weight in the RN treatment were greater than those in the Na treatment, although the differences were not significant. In contrast, leaf area significantly increased by 32.4% ($P < 0.05$, Fig. S1a–d). Inoculation with SiRMs

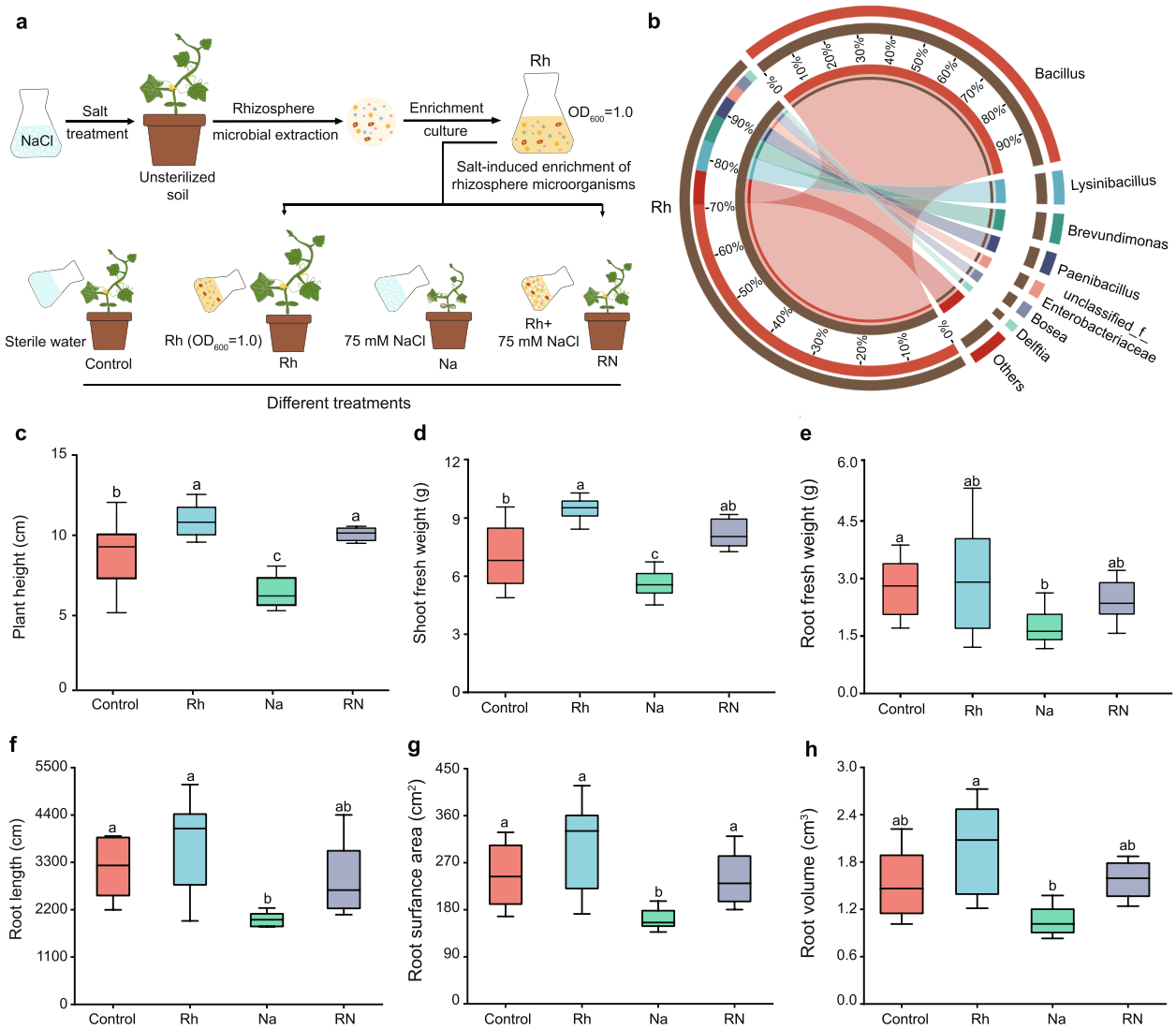

Fig. 2 Effects of salt-induced rhizosphere microorganisms (SiRMs) on the growth of cucumber seedlings under salt stress. **a** Schematic diagram of the experimental design. **b** Composition of salt-induced rhizosphere bacteria at the genus level. Effects of SiRMs on **c** plant height, **d** shoot fresh weight, **e** root fresh weight, **f** root length, **g** root surface area, and **h** root volume of cucumber seedlings under salt treatment. Duncan's multiple range test was used for multiple comparisons. Different letters denote significant differences between treatments ($P < 0.05$). All the plants were grown in sterile soil under four treatment conditions: treatment with sterile water (control), treatment with 75 mM NaCl (Na), treatment with SiRM inoculation under nonsaline conditions (Rh), and treatment with SiRM inoculation under 75 mM NaCl conditions (RN)

significantly promoted root growth under salt stress. Root length, root surface area, and root volume in the RN treatment increased by 47.9%, 48.2%, and 49.0%, respectively (Fig. 2f–h). These results indicate that SiRMs can alleviate the inhibition of cucumber seedling growth caused by salt stress by enhancing root development.

To further evaluate the effects of SiRMs, we measured key physiological parameters. Compared with physiological parameters in the Na treatment, SOD activity and root activity in cucumber seedlings in the RN treatment increased by 4.1% and 64.3%, respectively, whereas the POD activity and O_2^- decreased by 10.5% and 3.7%, respectively (Fig. 3a–d). Photosynthesis, stomatal conductance, and transpiration in the RN treatment were significantly greater than those in the Na treatment, increasing by 171.4%, 117.3%, and 95.3%, respectively. In contrast, the intercellular CO_2 concentration was significantly reduced by 17.6% ($P < 0.05$, Fig. S2 a–d). SiRM inoculation also significantly increased nutrient uptake under salt stress conditions. The ratios of P, K, Mg, Fe, Zn, and Mo in the RN group increased by 3.27-, 2.64-, 2.17-, 1.40-, 2.65-, and 1.79-fold, respectively (Fig. 3e). These results indicate that SiRMs improved the salt tolerance of cucumber seedlings by modulating the antioxidant system, enhancing root activity, and promoting nutrient absorption.

Salt-induced rhizosphere microorganisms maintain plant ion homeostasis under salt stress

To investigate the effect of SiRMs on the ion homeostasis of cucumber seedlings under salt stress, we measured the ion content in different parts of the plants, H^+-ATPase activity, and ion flux at the root tips. Compared with cucumber seedlings in the Na treatment, the Na^+ content in the roots of seedlings in the RN treatment was significantly lower by 15.8%, whereas the K^+ content in the roots increased significantly by 32.7% ($P < 0.05$, Fig. 4a–b). Although the Ca^{2+} content in the roots was greater in the RN treatment than in the Na treatment, the difference was not statistically significant. In the leaves and stems, the Na^+ content decreased by 9.7% and 18.9%, respectively, whereas the K^+ content increased

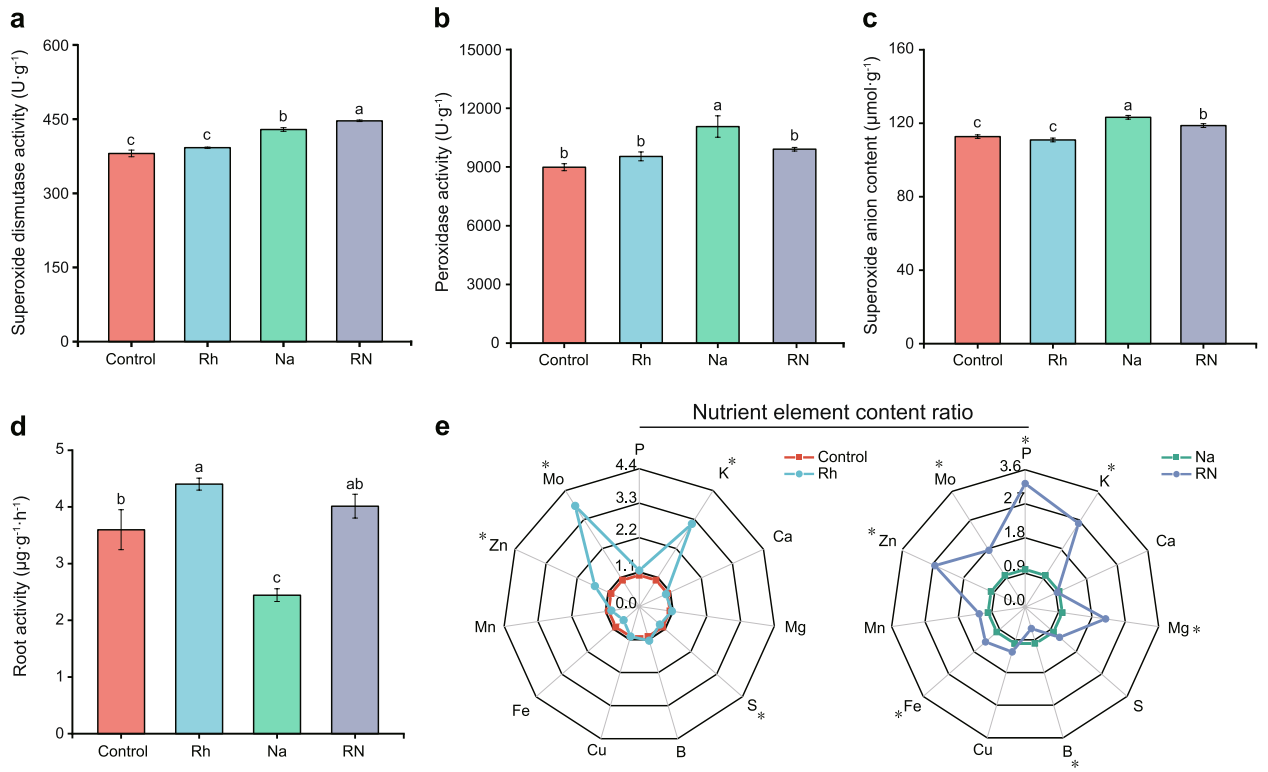

Fig. 3 Effects of salt-induced rhizosphere microorganisms (SiRMs) on the antioxidant enzyme activity, root activity, and nutrient uptake of cucumber seedlings under salt stress. **a** Superoxide dismutase (SOD) activity, **b** peroxidase (POD) activity, **c** superoxide anion (O_2^-) content, **d** root activity, and **e** nutrient element content ratio of cucumber seedlings in the rhizosphere microorganism and salt stress treatments. Duncan's multiple range test was used for multiple comparisons. Different letters denote significant differences between treatments ($P < 0.05$), and asterisks indicate significant differences at the 0.05 level. All the plants were grown in sterile soil under four treatment conditions: treatment with sterile water (control), treatment with 75 mM NaCl (Na), treatment with SiRM inoculation under nonsaline conditions (Rh), and treatment with SiRM inoculation under 75 mM NaCl conditions (RN)

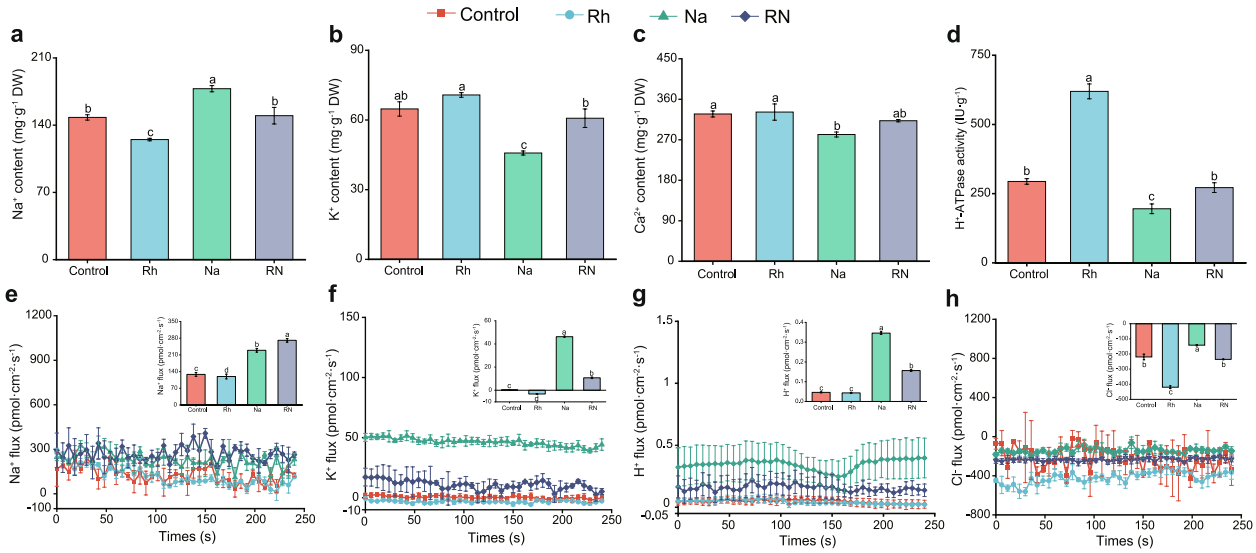

Fig. 4 Effects of salt-induced rhizosphere microorganisms (SiRMs) on the ion content in roots, root H^+-ATPase activity, and root ion fluxes of cucumber seedlings under salt stress. **a** Na^+ content, **b** K^+ content, **c** Ca^{2+} content, **d** H^+-ATPase activity, **e** Na^+ flux, **f** K^+ flux, **g** H^+ flux, and **h** Cl^- flux in the roots of cucumber seedlings in the rhizosphere microorganism and salt stress treatments. Positive values represent efflux, negative values represent influx, and different letters in the graphs indicate significant differences between treatments (Duncan's multiple range test, $P < 0.05$). All the plants were grown in sterile soil under four treatment conditions: treatment with sterile water (control), treatment with 75 mM NaCl (Na), treatment with SiRM inoculation under nonsaline conditions (Rh), and treatment with SiRM inoculation under 75 mM NaCl conditions (RN)

by 28.8% and 16.9%, and the Ca^{2+} content increased by 10.7% and 21.6%, respectively ($P < 0.05$, Fig. S3 a–f). Root H^+-ATPase activity in the RN treatment was significantly greater than that in the Na treatment and increased by 39.1% (Fig. 4d). The Na^+ efflux at the root tips in the RN treatment was significantly greater than that in the Na treatment and increased by 18.3%. Moreover, K^+ and H^+ efflux decreased by 76.7% and 54.8%, respectively, and Cl^- influx increased by 66.3% ($P < 0.05$, Fig. 4e–h). These results indicate that SiRM inoculation can alleviate the damage caused by salt stress by maintaining ion homeostasis in cucumber seedlings.

Salt-induced enrichment of rhizosphere microorganisms regulates gene expression in cucumber seedlings

To explore the mechanism by which SiRMs enhance salt tolerance, transcriptome sequencing was performed on the root systems of cucumber seedlings. Compared with the Na treatment, 1626 genes were significantly upregulated, and 1719 genes were significantly downregulated in the roots of cucumber seedlings in the RN treatment ($P < 0.05$, Fig. 5a–b). The top 20 GO enrichment terms for the RN treatment vs. the Na treatment are shown in Fig. 5c and include transmembrane transporter activity, transporter activity, secondary active transmembrane transporter activity, DNA-binding transcription factor activity, and transcription regulator activity, which are all related to ion transport. On the basis of the GO enrichment analysis results, the DEGs in the RN treatment vs. the Na treatment were categorized into three groups: biological processes, cell components, and molecular functions. Specifically, the biological processes were enriched in cellular processes, metabolic processes, and biological regulatory processes. The cellular components were enriched in the cell component, membrane component, and organelle terms. Molecular functions were enriched in catalytic activity, binding activity, and transport activity (Fig. 5d). These findings suggest that SiRM inoculation can regulate transcription in cucumber seedlings under salt stress, particularly in pathways related to ion transport.

Salt-induced enrichment of rhizosphere microorganisms affects the relative expression of ion transport genes in cucumber seedling roots

To verify the effect of SiRMs on ion transport in cucumber roots, we determined the transcription levels of genes related to ion transport. Compared with the Na treatment, the expression levels of genes encoding the Na^+/K^+ antiporter *CsHKT1* and high-affinity K^+ transporter *CsHAK5* were increased 7.79-fold and 4.20-fold, respectively, in the roots of cucumber seedlings in the RN treatment (Fig. 6a–b). The expression of the cation/H^+ transporter *CsCHX18;4* increased 5.37-fold, whereas that of *CsCHX18;1* and *CsCHX18;2* decreased 12.57-fold and 3.40-fold, respectively, in the roots of SiRM-inoculated

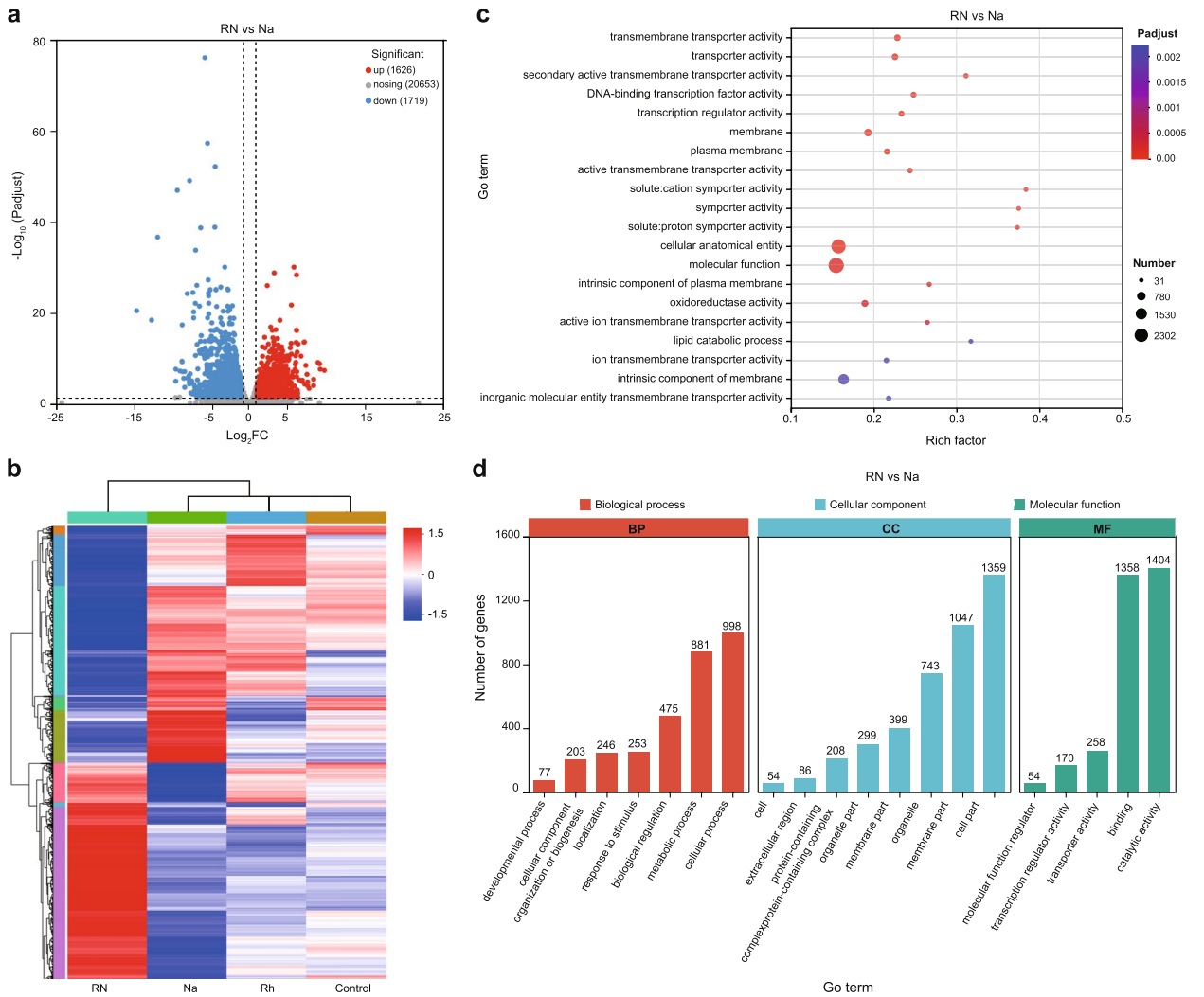

Fig. 5 Effects of salt-induced rhizosphere microorganisms (SiRMs) on gene expression in cucumber seedlings under salt stress. **a** Volcano plot of differentially expressed genes (DEGs) in the RN treatment vs. the Na treatment. **b** Heatmap and cluster analysis of DEGs under different treatments. **c** GO enrichment analysis of DEGs in the RN treatment vs. the Na treatment (top 20). **d** Functional annotation analysis of DEGs in the RN treatment vs. the Na treatment (top 20). BP: Biological process, CC: Cellular component, MF: Molecular function. RN: Cucumber seedlings inoculated with SiRMs under 75 mM NaCl conditions. Na: Cucumber seedlings treated with 75 mM NaCl

cucumber seedlings under salt conditions (Fig. 6c and Fig. S4 a–b). The expression of the sodium-calcium exchanger *CsNCX* decreased 2.56-fold in the RN group (Fig. 6d). Compared with the Na treatment, the expression of *CsHA3* and *CsCAX3* in the roots of cucumber seedlings in the RN treatment increased 28.32-fold and 42.03-fold, respectively (Fig. 6e–f). In the RN treatment, the expression of the ion channel proteins *CsCNGC14*, *CsCSC1*, and *CsMCU3* was significantly downregulated 5.34-fold, 5.07-fold, and 5.73-fold, respectively ($P < 0.05$, Fig. 6g–i). The expression of the sodium/potassium root-defective gene *CsNaKR1* increased 3.66-fold (Fig. S4 c). Conversely, the expression of *CsATL8* and *CsOCTN4* decreased 3.64-fold and 4.87-fold, respectively (Fig. S4 d–e). These results suggest that SiRMs promote K^+ absorption and reduce Na^+ accumulation by regulating the expression of ion transport genes in cucumber roots, thereby maintaining ion homeostasis in cucumber seedlings under salt stress.

Discussion

Salt-induced rhizosphere microorganisms play a pivotal role in promoting cucumber plant growth and enhancing salt tolerance. As soil salinity increased, specific rhizosphere microbial populations were selectively enriched around the plant roots. These microbes facilitated root

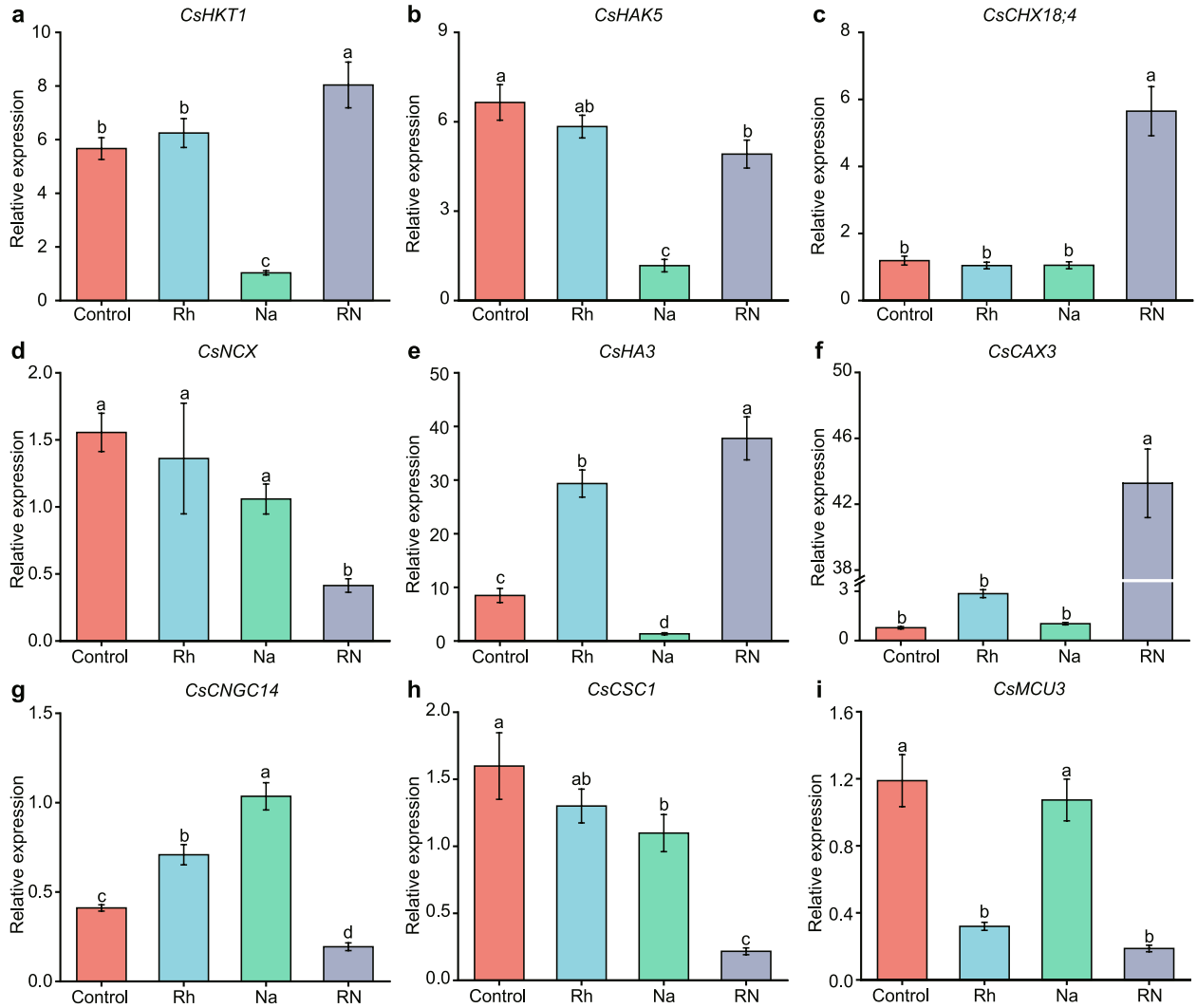

Fig. 6 Effects of salt-induced rhizosphere microorganisms (SiRMs) on the expression of genes related to ion transport in cucumber seedling roots under salt stress. For qRT–PCR analysis, *Actin* was used as the reference gene, and gene expression in roots without SiRM inoculation under 75 mM NaCl conditions was defined as 1. The data are presented as the means ± standard errors, and different letters on the graph indicate significant differences between treatments ($P < 0.05$)

system development by increasing the root surface area and absorption capacity. Simultaneously, they maintained intracellular ion homeostasis through multiple pathways, which increased plant salt tolerance. Furthermore, SiRMs regulate the expression of genes related to ion uptake and transport in plant roots. Our results demonstrate that SiRMs can help plants maintain ion homeostasis and promote plant root development salt tolerance.

Resistance to abiotic stress is not determined solely by the plant genome but is also influenced by symbiotic microorganisms that extend the adaptive capabilities of plants [38, 39]. In this study, we analysed the composition of microbial communities in different locations in seedlings from control and salt-stressed treatments to characterize the microbial community structure. Our results revealed that the soil microbial community, particularly the rhizosphere bacterial community, responded positively to salt stress, and the dominant phylum was *Proteobacteria* (Fig. 1a). Moreover, the root endophytic bacterial community differed significantly from the bacterial communities in the rhizosphere soil and bulk soil (Fig. 1b), likely due to selective filtering processes of the plant root system [40]. Canonical phylogenetic correspondence analysis (CPCoA) revealed a significant separation between the microbial communities in the control and salt-stressed treatments (Fig. 1c), which indicates that salt stress altered microbial community diversity. This change may be attributed to an increase in soil

electrical conductivity caused by salt stress, which subsequently affects microbial abundance [41]. Functional predictions for microbial communities included chemoheterotrophy, nitrate reduction, and aromatic compound degradation, all of which are the main functions of microbial communities. Degradation (aromatic compound degradation) and urea degradation (ureolysis) are closely associated with nutrient uptake cycles [42, 43].

Salt stress impairs plant growth. Recent studies have shown that plants in specific environments recruit beneficial microbes to increase stress resistance [44, 45]. Plants benefit more from diverse microbial communities than from single microbial interactions [46]. Sequencing has revealed that salt stress enriches rhizosphere microorganisms, particularly *Bacillus* species (Fig. 2b), which are known for their high salt tolerance [47, 48]. Our results indicated that inoculation with SiRMs significantly alleviated the inhibition of growth in salt-stressed cucumber seedlings; plant height and aboveground and belowground fresh weights increased (Fig. 2c–e). Additionally, inoculated roots presented greater surface area, length, and volume than noninoculated controls did (Fig. 2f–h). Abdelkefi et al. [49] demonstrated that several bacilli isolated and screened from the plant rhizosphere can promote the growth of tomato seedlings under salt stress and increase the salt tolerance of tomato plants. In this study, salt stress reduced the leaf area (Fig. S1d) and impaired photosynthesis (Fig. S2). Rates of photosynthesis, stomatal conductance, and transpiration were significantly lower under salt stress but improved with the inoculation of SiRMs (Fig. S2). Consistent with our findings, previous studies have reported that inoculation with PGPR improves photosynthetic efficiency in cucumber seedlings under salt stress by modifying the chloroplast structure and increasing the chlorophyll content [50]. Salt stress also affects antioxidant enzyme activity, but microorganisms can mitigate this damage by activating plant antioxidant systems. Cucumber seedlings under salt stress had elevated levels of SOD and POD (Fig. 3a, b) that were likely due to plant responses. Compared with seedlings in the control treatment, the antioxidase activity of the cucumber seedlings inoculated with SiRMs was not significantly affected, which may be due to the absence of external stress. Elevated enzyme activity is crucial for scavenging ROS and improving plant resistance [51, 52], and cucumber seedlings inoculated with SiRMs presented greater SOD activity and lower $O_2 \cdot^-$ contents under salt stress (Fig. 3c), which suggests that these microorganisms can alleviate damage from salt stress. These results corroborate the findings of Siddika et al. [53].

Notably, the rhizosphere microbial community also exhibited significantly increased root vigour, which suggests that inoculation with SiRMs can enhance root function [54], improve nutrient uptake capacity, and promote plant development [55]. Essential nutrients are critical for plant growth and yield, and nutrient deficiency can negatively impact plant development [56, 57]. Under saline conditions, elevated Na^+ and Cl^- limit the uptake of many nutrients [14], which inhibits normal plant growth and development [7]. Rhizosphere microorganisms have been shown to promote nutrient absorption. For example, *Pseudomonas*, *Bacillus* [58, 59], and *Trichoderma* [60, 61] secrete Fe carriers and decompose P and K, which increases the availability of mineral elements in soil and promotes nutrient absorption by plants under salt stress [58, 62]. In this study, rhizosphere microbial suspensions significantly increased the uptake of P, K, Mg, Fe, Zn, and Cu in plants under salt stress (Fig. 3e). These findings indicate that SiRMs promote nutrient uptake and assist plants in resisting the harmful effects of salt stress.

Ionic homeostasis is essential for plant growth and environmental adaptation. Under salt stress, plants require high K^+/Na^+ ratios for normal cellular processes [63, 64]. In this study, the Na^+ content was significantly lower and the K^+ content was significantly higher in the roots of plants inoculated with SiRMs under salt stress, and Ca^{2+} increased nonsignificantly (Fig. 4a). The Ca^{2+} content was significantly higher in leaves and stems (Fig. S3 a, b). A decrease in K^+ under salt stress has been attributed to high Na^+ concentrations that hinder the activity of K^+-specific transporter proteins, which leads to K^+ efflux [65, 66]. Maintaining intracellular ion homeostasis under salt stress is crucial for plant growth and development [67–69]. We found that the Na^+ efflux rate and Cl^- uptake rate were significantly higher, whereas the K^+ and H^+ efflux rates were significantly lower in plants inoculated with SiRMs under salt stress (Fig. 4c–e). Cl^- can promote plant growth by increasing water utilization, regulating leaf cell size, and increasing photosynthesis [70–72]. Enhanced H^+-ATPase activity facilitates Cl^- and K^+ [73, 74]. Cl^- may function as an auxiliary ion for K^+ and play a significant role in maintaining ion homeostasis [75].

HKT1 and *HAK5* are primary transporter proteins responsible for the uptake of K^+ in the root system [76, 77]. Salt stress inhibits their expression, but SiRMs significantly increase their expression under salt stress (Fig. 6a, b Fig. S4 a) and reduce Na^+ influx through the plasma membrane [78, 79]. *CHX* on the tonoplast mediates K^+, Na^+, and H^+ transport and maintains pH homeostasis [80, 81]. *CsCHX18;1* and *CsCHX18;2* were downregulated in response to the treatment with 75 mM NaCl and SiRMs (Fig. S4 b, c) and likely protected plants by promoting osmotic substance accumulation [82]. Conversely,

CsCHX18;4 was significantly upregulated in the treatment with SiRMs (Fig. 6c), which promoted the absorption of K^+ and maintained the ion balance in plants [83]. The Na^+ content in cucumber plants increased in the salt treatment, possibly due to *NCX* (Na^+/Ca^{2+} exchanger) activity, which sequesters Na^+ into vacuoles while releasing Ca^{2+} into the cytoplasm [84]. *CsNCX* expression decreased significantly after inoculation with SiRMs under 75 mM NaCl (Fig. 6d). *HA3* expression is inhibited by salt stress but increased after inoculation with SiRMs under 75 mM NaCl (Fig. 6e), which improved H^+-ATP activity and promoted Na^+ efflux [85]. *CAX3* (a cation/proton exchanger) plays an important role in regulating the homeostasis of metal ions (Fe^{2+} and Zn^{2+}) [86]. Its expression increased significantly after inoculation with SiRMs under 75 mM NaCl (Fig. 6f), and this reduced ROS and activated the antioxidant oxidase system, increased salt tolerance [87], and promoted H^+-ATPase activity in the plasma membrane [88, 89].

As a class of nonselective cation channels, *CNGCs* can be activated by Na^+, K^+, and Ca^{2+} [90–92]. Salt stress induced *CsCNGC14* expression was downregulated by SiRMs under 75 mM NaCl (Fig. 6g), which inhibits Na^+ absorption [93]. *CSC1*, a calcium osmotic stress-gated cation channel of the *OSCA* family, is activated by high osmotic pressure from Na^+ accumulation under salt stress [94]. However, *CsCSC1* expression was reduced by SiRMs under 75 mM NaCl (Fig. 6h). *MCU3* (mitochondrial calcium unidirectional transporter) expression is induced by salt stress [95] and may lead to excessive mitochondrial Ca^{2+} and stress damage. SiRMs significantly reduced *CsMCU3* expression under 75 mM NaCl ($P < 0.05$) (Fig. 6i).

CsNaKR1 expression in plants in the salt stress treatment was lower than that in plants treated with SiRMs under 75 mM NaCl (Fig. S4 d). Loss of *NaKR1* leads to the accumulation of Na^+ [96]. *ATL8*, a RING E3 ligase, plays an important role in the regulation of phosphate homeostasis. We found that inoculation with SiRMs under 75 mM NaCl significantly promoted P uptake (Fig. 3e and Fig. S4 e). *CsOCTN4* expression is induced by salt stress but reduced by SiRMs under 75 mM NaCl (Fig. S4 f), and ion homeostasis is maintained by reducing cytoplasmic Na^+ [97]. These genes facilitate the absorption of K^+ and Ca^{2+} and promote Na^+ efflux through positive and negative regulation, thereby maintaining ion homeostasis and enhancing plant salt tolerance.

Conclusion

Salt stress markedly alters the composition, structure, and function of the rhizosphere microbial community, which severely impedes the growth of cucumber seedlings. However, inoculation with SiRMs can significantly increase nutrient absorption under salt stress conditions and reduce the Na^+/K^+ ratio, thereby mitigating damage from salt stress and supporting plant growth. Rhizosphere microorganisms also modulate the expression of ion transport-related genes, including K^+ transporters, cation/H^+ antiporters, and ion channel genes, in cucumber seedling roots under salt stress. These findings confirm the ability of SiRMs to increase salt tolerance in plants and increase our understanding of microbial-assisted salt tolerance mechanisms in plants, thus providing valuable insights for future research on salt tolerance in crop plants.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06699-0.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.
Supplementary Material 8.
Supplementary Material 9.

Authors' contributions

YW, HX, and HL developed the study concept and experimental design. HX and HL supervised the project. YW, YG, CL and XS performed laboratory work. YW, YG, MY and WL collected the samples. YW and HL conducted data analysis. YW and HL wrote and revised the manuscript. All authors read and approved the final version of the manuscript.

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Data availability

All data generated or analyzed in the course of this study may be obtained from the corresponding author upon reasonable request. All raw amplicon reads can be found in the NCBI (National Center for Biotechnology Information) database. The SRA accession numbers for the microbiome and transcriptome are PRJNA1163060 and PRJNA1163670, respectively.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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