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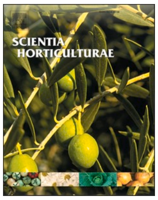

Research Paper

The Enterobacter sp. SA187 stimulates stress-responsive genes and promotes salt and heat stress tolerance in tomato plants

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ABSTRACT

Enterobacter sp. SA187, is a plant growth-promoting bacterium (PGPB) with potential to enhance abiotic stress tolerance in various crops. This study evaluated the impact of inoculation of SA187 to salinity and heat stress tolerance of *Solanum lycopersicum* L, tomato. Different alterations in the physiological, biochemical and molecular responses against the salt and heat stress due to the beneficial association of tomato plants with SA187 were investigated. Colonization by SA187 significantly enhanced tomato plants growth under both saline and heat stress condition. It induced an enhancement of $>$ 90 % in the morphological and physiological processes resulting in an increased root hair growth and higher plant biomass. The inoculation also caused a 65 % decline in Na^{+}/K^{+} ratio, increased chlorophyll content and improved the antioxidant enzyme activity of Superoxidase dismutase (SOD), Peroxidase (POD), and Catalase (CAT), particularly under salt and heat stress conditions. The RNA-seq analysis produced clean reads that ranged from 33,812,161 to 44,114,539 across the different groups of treatment, which were successfully assembled into 35,825 annotated genes. Transcriptomic analysis of the genes involved in the enhancement of the physiological and biochemical processes revealed at least a 2-fold increase in the expression of *NHX3* and *ERF4* in leaves, *SOS2, SOS4*, and *SULTR3;5* in leaves and roots. For heat stress, SA187-colonized tomato plants displayed higher expression of heat shock protein genes in leaves such as *HSP17.4B, HSP17.6, HSP22.0*, and *HSP26.5*. These findings show the possibility of using the Enterobacter sp. SA187 as a sustainable solution for enhancing crop tolerance and productivity in challenging environmental conditions.

1. Introduction

Plants productivity and survival are threatened by abiotic stresses. Among these, salinity and heat stresses are expected to be significantly increased in arid regions due to climatic changes, transforming cropping systems and biota, leading to a decline in food production (Benitez-Alfonso et al., 2023). Salinity mainly affects crops through ionic and osmotic stress (Kibria and Hoque, 2019). This occurs due to excessive sodium (Na^{+}) accumulation that disrupts cellular homeostasis, leading to reduced water uptake, disrupted plant cell growth and increased production of reactive oxygen species (ROS), which affects both plant yield quality and quantity (Albacete et al., 2008).

Solanum lycopersicum (tomato) is a commonly consumed fresh or processed food product (Roșca et al., 2023). Salinity impacts all aspects of the growth of tomato plants. It exhibits a range of physiological and biochemical responses to reduce ionic toxicity and osmotic imbalance that result from salinity. Genetic variations in *SlSOS1*, a plasma membrane Na^{+}/H^{+} antiporter mediating Na+ extrusion in root epidermal cells to reduce Na+ accumulation, was found to contribute to the phenotypic variation of salt tolerance in tomato (Wang et al., 2021). Additionally, osmoprotectants like proline accumulate in tomatoes to stabilize proteins and membranes, ensuring cellular function under saline conditions (Ahanger et al., 2020). Antioxidant enzymes in tomatoes, including superoxide dismutase (SOD) and catalase (CAT), play a crucial role in scavenging ROS produced due to salt-induced oxidative stress.

Short-term heat exposure causes leaves to reorient, undergo osmotic

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adjustments, evaporate, and change their cell membrane structures (Bäurle, 2016; Zhong et al., 2024). Tomato plants are highly susceptible to heat stress, which adversely affects growth, fruit set, and yield by disrupting photosynthesis and reproductive development. A response to high temperatures in tomatoes is the upregulation of heat shock proteins (HSPs), such as HSP70 and HSP90, which act as molecular chaperones to protect proteins from denaturation (Graci and Barone, 2024). Additionally, tomatoes enhance antioxidant activity through increased expression of ascorbate peroxidase (APX) to prevent oxidative damage caused by heat-induced ROS accumulation (Lee et al., 2023).

Presently, plant growth-promoting rhizobacteria (PGPR) have emerged as a promising solution to enhance plant tolerance to abiotic stress. Numerous studies have demonstrated the effectiveness of PGPR as biological agents for the management of crop plants against drought and salt stress, thereby improving plant growth and production (Vafa et al., 2021; Nishu et al., 2022; Zhao et al., 2022). These beneficial bacteria utilize various strategies to support plant development, including enhancing nutrient absorption, synthesizing plant hormones, making insoluble minerals available for plants, fixing nitrogen, suppressing diseases, stimulating root growth, and improving soil structure (Timofeeva et al., 2023). The incorporation of PGPR into agricultural ecosystems is a crucial aspect of sustainable farming practices, as it can reduce dependence on artificial fertilizers and pesticides, while boosting plant growth and soil health (Muhie, 2022). Recent studies have focused on harnessing the potential of endogenous microbiomes from desert plants as biofertilizers to enhance crop productivity and stress tolerance (Kumar et al., 2022; Dhar et al., 2024). One particularly promising plant growth-promoting bacterium (PGPB), the Enterobacter sp. SA187 isolated from the root nodules of *Indigofera argentea*, a native desert plant found in the Jizan region of Saudi Arabia, has shown remarkable potential in enhancing multi-stress tolerance in both alfalfa crop plants under desert farming conditions and the model plant Arabidopsis (de Zélicourt et al., 2018).

Upon colonization, SA187 undergoes significant changes in gene expression related to chemotaxis, flagellar biosynthesis, quorum sensing, and biofilm formation (Synek et al., 2021). This bacterium reprograms sulfur metabolism in Arabidopsis plants, helping them overcome salt stress-induced sulfate starvation and partially rescuing salt hypersensitivity in sulfur metabolism mutants (Andrés-Barrao et al., 2021). Furthermore, SA187 induces thermotolerance in wheat and Arabidopsis through ethylene signaling and HSFA2-dependent enhancement of H3K4me3 levels at heat-stress memory gene loci (Shekhawat et al., 2021). Unlike thermopriming, SA187 induces constitutive chromatin modifications, resulting in robust thermotolerance.

Solanum lycopersicum L., tomato is the world's most cultivated solanaceous vegetable crop. However, domesticated varieties have triggered a loss in salinity and heat stress tolerance which negatively affected their growth and reduced fruit production (Gálvez et al., 2012; Pailles et al., 2020; Camejo et al., 2005; Driedonks et al., 2018). A potential solution to overcome this problem is the utilization of Enterobacter sp. SA187. In this study, we investigated the colonization of tomato roots with the Enterobacter sp. SA187, an endophytic bacterium from desert plant. We found that inoculation of SA187 enhances tomato plant growth and resistance to salt and heat stress under greenhouse conditions. By performing physiological, biochemical and transcriptomic analyses, our results revealed that the colonized tomato plants induced plant stress tolerance.

2. Materials and methods

2.1. Preparation of Enterobacter sp. SA187 culture and seed coating

The bacterial strain used in this study, Enterobacter sp. SA187 was provided by Professor Hirt from the King Abdullah University of Science and Technology (KAUST), Saudi Arabia. This strain was originally isolated from the root nodules of *Indigofera argentea* and has been characterized for its plant growth-promoting abilities. It was stored at –80 °C in 50 % glycerol prior to use. Enterobacter sp. strain SA187 was cultured by inoculating a single colony into lysogeny broth (LB) and raised overnight at 37 °C with 200 rpm in a rotary shaker until a cell concentration of 10^6 CFU/ml was obtained. *S. lycopersicum* seeds was purchased from a local store, surface-sterilized by shaking for 10 min in 1 ml of 70 % EtOH and 0.05 % sodium dodecyl sulfate (SDS) and washed three times with 1 ml of Milli-Q sterile water. Sterile seeds were placed in Erlenmeyer flasks containing the SA187 culture medium and incubated for 1 h at 37 °C in a shaker. The seeds were dried and sown in sterilized peat moss (BVB Peat moss potting soil).

2.2. Salinity and heat stress treatments of tomato seedlings

After three weeks of germination, the tomato plants cv. Roma were transplanted into individual peat moss pots and left to grow for another two weeks under controlled greenhouse conditions. The optimal day temperature for tomato under the greenhouse was in the range of 21 °C–24 °C, and a nighttime temperature of approximately 16 °C–18 °C. Relative humidity was kept between 60 % and 70 %, and plants received a 16 h light photoperiod. The treatment groups were subjected to salt stress by placing their respective pots in trays filled with 200 mM NaCl for 15 days, knowing that the growth and morphological characteristics of tomato plants will decline with the exposure of 100 mM to 200 mM NaCl (Shiyab et al., 2013). The control and treatment groups were separated to avoid cross-contamination with the bacterial strains. After 15 days, plant leaves and roots were collected and kept at -80 °C. Separately from the salt stress experiment, the impact of SA187 on the tomato heat stress tolerance was investigated by stressing the plants at 42 °C for 2 h in a growth chamber. After heat stress, plant leaves were collected immediately and kept at -80 °C for until RNA extraction was performed. Additionally, the fresh weights of the leaves and roots were measured to determine whether SA187 affected the biomass under stress.

2.3. Physiological and biochemical characterization of tomato plants under salinity and heat stress

After applying the stresses of salinity and heat, leaves of the plants were harvested in triplicate and used for downstream analysis, including the measurement of physiological and biochemical parameters such as the Na^+/K^+ ratio, chlorophyll content, and activities of different antioxidant enzymes, which included Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT).

2.3.1. Measurement of the Na^+ and K^+ ions concentrations

To quantify the concentration of Na^+ and K^+ ions in tomato plant samples, leaves and roots from both inoculated and non-inoculated seedlings, grown under normal (control) and salt stress conditions, were collected in triplicate. These samples were oven-dried at 80 °C for 24 h. The dried tissues were then fully digested in 1 % HNO_3 at 60 °C overnight to extract the ions. The concentrations of Na^+ and K^+ ions in the solution were determined using an inductively coupled plasma optical emission spectrophotometer (ICP-OES).

2.3.2. Determining the total chlorophyll content

To measure the chlorophyll constituent, fresh leaves were harvested in triplicate from the treatments. The weight of the leaves was measured and were crushed with 80 % acetone solution in a mortar and to extract leaf chlorophyll. The extract was centrifuged to remove debris and insoluble particles. The absorbance of the supernatant was measured at 646 and 663 nm (Munns and Tester, 2008). The formula used to calculate the total chlorophyll content was $[(7.15 \times OD_{663}) + (18.71 \times OD_{646})]$ V/M, wherein OD_{663} and OD_{646} are the absorbance values at the respective wavelengths, V indicates the volume of leaf, and M shows the fresh material weight. The resulting value was expressed in mg g^{-1} (FW), which represents the amount of chlorophyll present in the sample per gram of fresh material.

2.3.3. Antioxidant enzymatic assays

Fresh leaves were collected from control and SA187-colonized tomato plants under both stress and non-stress conditions. The leaves were crushed into fine particles in mortar and pestle with the use of liquid nitrogen. The sample was homogenized in a buffer constituting 0.1 M potassium phosphate with the pH maintained at 7.0, supplemented with polyvinylpolypyrrolidone (PVPP) (0.1 g), 0.5mM PMSF (phenylmethyl sulfonate fluoride), and EDTA (0.1 mM). The mixture was centrifugated, and the supernatant was utilized for enzyme activity assay. Protein concentrations in the various extracts were analyzed using bovine serum albumin (BSA) as the gold standard following Bradford's protocol (Bradford, 1976). To measure CAT activity, the method of Aebi (1984) was adopted (Aebi, 1984), in which the disappearance of hydrogen peroxide was monitored at OD = 240 nm. The reaction solution was prepared in a 2 ml volume, consisting of 0.1 M potassium phosphate buffer with the pH at 7.0, 100 µl of the extracted enzyme, 0.1 mM EDTA, and 20 mM H_2O_2. The activity of SOD was measured by estimating its ability to slow the photochemical reduction of nitro blue tetrazolium using Beyer and Fridovich's method (Beyer and Fridovich, 1987). The enzyme quantity leading to half inhibition of NBT reduction at 25 °C was estimated to be one unit of SOD. The levels of SOD are expressed in units min^{-1} mg^{-1} protein. The POD activity was determined using the guaiacol test, which measures the absorbance change at 470 nm, following the method of Polle et al. (1994). The POD activity assay was conducted over 3 min using a reaction mixture containing a buffer of 100 mM potassium phosphate with the pH 7.0, 0.1 ml of enzyme extract, 10 mM H_2O_2, and 20 mM guaiacol, prepared in a total volume of 3 ml.

2.4. Extraction of total RNA and preparation of cDNA libraries for RNA sequencing

After two weeks of salt stress, roots and leaves in triplicates from SA187 colonized and non-colonized tomato plants were collected separately from the salt-stressed and control plants. For the heat stress experiment, only leaves were collected immediately after 2 h of heat stress at 42 °C from SA187 colonized and non-colonized plants. Total RNA was extracted in triplicate from tomato leaf and root samples of various treatment groups through the Spectrum Plant Total RNA Kit of Sigma, with the manufacturer's protocol. RNA concentration was determined using a NanoDrop1000 spectrophotometer (Thermo Scientific, USA). RNA from each sample was pooled, and around 3 µg per sample RNA was used for cDNA synthesis. The cDNA libraries were prepared with the TruSeq RNA Sample Preparation Kit (Illumina, USA), following the manufacturer's instructions. Indexed codes were incorporated to associate sequences with their corresponding samples. Paired-end sequencing for all libraries was performed by BGI (China) on the Illumina HiSeq platform.

2.5. Bioinformatics analysis of RNA-seq data

Quality filtering procedures were applied to examine the raw data in fastq file format. The fastp tool was employed to filter out adaptor sequences and low-quality reads (quality scores below Q20), producing high-quality clean reads for subsequent analyses. For each sample, the tomato reference genome and corresponding gene annotation files were retrieved from https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000188115.5/ (Assembly accession: GCF_000188115.5). The reference genome was indexed using HISAT2 (v2.0.5) software (Kim et al., 2015), and the paired-end clean reads were matched to the reference genome. Transcript abundance and read counts were quantified using a feature counting tool (Liao et al., 2014). Gene expression levels were evaluated by calculating fragments per kilobase of transcript sequence per million base pairs (FPKM), a metric that normalizes expression based on transcript length and the total number of mapped reads. Differentially expressed genes (DEGs) between control and treatment conditions were identified based on a 2-fold change threshold and a P-value < 0.05. Differential expression analysis was performed using the DESeq2 R package (version 1.43.1) (Love et al., 2014). Gene expression analysis was conducted by calculating the fold-change in the expression of the target genes between the treatment and control groups. The fold change was determined as the ratio of gene expression in the treatment group relative to that in the control group using the following formula: Fold Change = Expression treatment/expression control. A fold-change greater than 1 indicated upregulation of the gene in response to treatment, while a fold-change of less than 1 indicated downregulation. This analysis allowed the identification of genes that were differentially expressed as a result of the treatment compared with the control condition. Finally, AgriGO (Du et al., 2010) was employed to identify associated GO keywords (FDR 0.05) and functions of the relevant genes.

2.6. Quantitative real-time PCR for the stress-responsive gene expression analysis

RNA

RNA was extracted from the roots and leaves of both control and stressed tomato plants, including SA187-colonized and non-colonized samples, with the RNeasy Total RNA Isolation Kit (Qiagen). For cDNA synthesis and the removal of any residual genomic DNA contaminants, 1 µg of total RNA was treated with RNase-free DNase I (Invitrogen) at 37 °C for 30 min to ensure complete degradation of DNA. Following DNase treatment, the RNA was purified using a cleanup step to eliminate enzyme traces and reaction byproducts. This was then converted into cDNA through the use of the SuperScript III Reverse Transcriptase Kit (Invitrogen), following the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was performed with Applied Biosystems SYBR^{TM} Select Master Mix in 96-well plates the qRT-PCR system.

PCR reactions were carried out in a total volume of 10 µl, consisting of 3 µl of cDNA, 0.5 µl of 10 µM primers (forward and reverse), 5 µl of 2 × SYBRTM Select Master Mix, and 1 µl of RNase-free water (Sigma). The thermal cycling protocol began with an initial denaturation for 10 min at 94 °C, succeeded by 45 cycles at 94 °C for 10 s, 52 °C for 10 s, and 72 °C for 15 s. A melting curve analysis was subsequently performed, starting at 95 °C for 5 s, 65 °C for 1 min, and gradually increasing the temperature from 65 °C to 97 °C over 5 min. Primers for qRT-PCR were constructed using Primer3 software, as outlined in Table 1. The relative gene expression levels were determined using the $2^{-\Delta\Delta CT}$ method, in which ΔΔCT represents the difference in (CT of the target gene – CT of the actin gene) under stress conditions compared to (CT of the target gene – CT of the actin gene) under normal conditions.

2.7. Statistical analysis

Statistical metrics, including the mean and standard deviation (SD), were calculated to evaluate the responses of both colonized and non-colonized tomato plants under normal and stress conditions (salinity and heat). Each experiment included three biological replicates. Data distribution was assessed using the Shapiro-Wilk test to confirm normality, while Levene's test was applied to measure the homogeneity of variances. After confirmation of the data, a one-way analysis of variance (ANOVA) was conducted using a completely randomized design to compare the effects of SA187 inoculation under control and stress conditions. Post-hoc comparisons were performed using Tukey's HSD test at a significance level of 0.05. The data has been analysed using the IBM SPSS Statistics 30.0.0 (IBM Corp., Armonk, NY, USA).

Table 1
Primer sequences for the analysis of gene expression in tomato plants.

Genes	Primer sequences (5′-3′)	Accessions
Actin 7	Forward: CCAACAGAGAGAAAATGACCC Reverse: CCAGAGTCCAACACAATACC	Solyc11g005330.2.1
NHX3	Forward: AAGAACGCCGCCAATAAC Reverse: TCAAGAAGCCAAAAACCAGA	Solyc10g006080.2.1
SOS2	Forward: CGATTTATTTCCCGCCAACC Reverse: TTGACCAGCCCTATTTGCC	Solyc12g009570.2.1
SOS4	Forward: TCACACAGGCAAACCCAAC Reverse: GCTTACCTTCATCACCCATCAC	Solyc02g091340.3.1
LEA2	Forward: ACCCTTACTCTGTTCCTATTCC Reverse: TCATTTGCCTTTATGCTCCC	Solyc01g095150.3
ERF4	Forward: TCACCCAATTCCTCATACCC Reverse: AAATCCTCGATCCTTTTCGAC	Solyc05g052030.1
SULTR3;5	Forward: CCCAAACACTTCAAACACCC Reverse: AAACCAGCCAAGATGTCCC	Solyc04g054730.3
AUX	Forward: ACAACTCTCTCATTCTTTGACC Reverse: TCCAATCCCCATCCTTATCTTC	Solyc06g053830.3.1
HSP17.4B	Forward: CCAACTTCTCTTTCCAGAATCC Reverse: TCTCATCTTCCACACTCACC	Solyc03g123540.3.1
HSP17.6	Forward: ACACACCACTCTTCCACAC Reverse: TCTCTTCTTCCCTCTTCCTTTC	Solyc08g062450.1.1
HSP22.0	Forward: CAGGTATCATCACTAAGCGCAG Reverse: TTCGCCGTCTCTTTCCAGTC	Solyc11g020330.1.1
HSP26.5	Forward: AGAAACAACAAGTAGATGGCAG Reverse: GACAAGAGCAGGTGCAAAG	Solyc12g042830.2.1

3. Results

3.1. *Enterobacter sp. SA187 colonization and effect on tomato plants*

Colonization with the Enterobacter SA187 significantly increased tomato plant growth under both saline and heat stress conditions, outperforming untreated control plants. This enhancement was observed through morphological alterations in tomato plants, that resulted in an increased growth in vegetative plant parts (Fig. 1a) and the formation of broader root (Fig. 1b). Under 200 mM salt stress, SA187 considerably enhanced the leaf fresh and dry weights, specifically resulting in an increase of 126.29 % of leaf fresh weight (Fig. 1c) and 97.06 % of dry weight (Fig. 1d).

Similarly, under 42 °C of heat stress, an enhancement of 134.53 % was observed in the leaf fresh weight and 68.21 % in the leaf dry weight with SA187 inoculation of shoots and roots, respectively. Furthermore, SA187 inoculated plants displayed more than 150 % increase in the root fresh and dry weights (Fig. 1d and e) under the salt stress condition. Moreover, a significant difference was found in the leaf dry weight, root fresh weight, and root dry weight of SA187 inoculated plants under salt and heat stress, with salt stress SA187 inoculated plants exhibiting higher responses.

3.2. *SA187 colonization regulates Na^+ and K^+ homeostasis in tomato plants*

The Na^+/K^+ ratio was examined in tomato leaves under the control and salt stress. In the control condition, both leaf and roots showed no significant difference in the Na^+/K^+ ratio of SA187 inoculated and non-inoculated tomato plants (Fig. 2a). Whereas, under the salinity stress condition, we found a substantial decrease in the Na^+/K^+ ratio of SA187 colonized plant leaves (0.62) and roots (0.83) compared to non-inoculated plant leaves (1.77) and roots (1.91) (Fig. 2a). This shows a reduction of 65 % in leaves Na^+/K^+ ratio of SA187 inoculated plant leaves and 57 % in roots compared to non-inoculated plants. In addition, Na^+/K^+ ratio of SA187 inoculated plants under salt and heat stress remained similar.

Chlorophyll content showed a significant difference between SA187 inoculated and non-inoculated plant leaves in the conditions of control, salt, and heat stress. The highest chlorophyll content was observed for the SA187 colonized plant under control condition (2.33 mg g^{-1} FW). In addition, the SA187 inoculation led to a significant increase in chlorophyll levels with 41.55 % of increase under the salt stress condition (Fig. 2b). Similarly, SA187 colonization resulted in significant increase of 65.39 % in the heat stressed plant leaves. This suggests a role of Enterobacter sp. SA187 in enhancing photosynthesis and plant vitality under normal and abiotic stress condition. The chlorophyll content of SA187 colonized plants under salt and heat stress showed difference, with more chlorophyll content in the heat stress SA187 inoculated plants.

3.3. *Tomato plants exposed to salt and heat stress exhibited increased antioxidant enzyme activities when colonized with SA187*

The activities of SOD, POD, and CAT antioxidant enzymes showed no significant difference under control condition. However, they differed significantly in comparison to the salinity and heat stress condition. Under the salt stress condition, SOD activity was observed to be 1.18 Unit min^{-1} mg^{-1} protein, while for SA187 it showed an enhancement of 26.27 %. Similarly, 20.45 % of increase in SOD was found under the heat stress condition (Fig. 3a). In addition, a significant difference was observed in the POD activity under both salinity and heat stress conditions. The POD activity was enhanced in the SA187 colonized tomato plants under salt stress condition, showing an increase of 14.67 % (Fig. 3b). While, under the heat stress condition the colonization with SA187 resulted in an improvement of 20.28 % in the POD activity. The highest POD activity was observed in SA187 plant leaves under salt stress (Fig. 3b). Furthermore, the non-colonized and SA187 colonized plants under normal condition displayed similar CAT activity, indicating no significant changes (Fig. 3c). The salt treatment significantly increased CAT activity with 18.9 %, showing a strong response of SA187 to salinity stress. Similarly, SA187 colonized plants under heat stress showed a significant increase of 43.38 % in the CAT activity (Fig. 3c). The CAT activity was observed to be highest under the heat stress plants colonized with SA187.

3.4. *RNA-seq of tomato plants under salt and heat stress*

3.4.1. *Read count distribution and gene dispersion distribution*

cDNA libraries were generated using RNA isolated from the roots and leaves of SA187 colonized and non-colonized salt- and heat-stressed tomato leaves to investigate the molecular basis of salt and heat tolerance and adaptation at the transcriptomic level. The RNA-seq analysis produced clean reads that ranged from 33,812,161 to 44,114,539 across the different groups of treatment, which were successfully assembled into 35,825 annotated genes. Based on the quality score and read length, more than 85 % of clean reads were mapped to protein-coding genes (Fig. 4a).

The PCA plot illustrates gene expression variance under different experimental conditions, emphasizing the effects of salt and heat stress with and without SA187 colonization (Fig. 4b). The SA187 colonized plant leaves under heat stress emerged as an outlier, showing significant transcriptional changes in leaves due to bacterial inoculation under heat stress. SA187 colonized plant roots under control and salt stress conditions exhibited distinct expression patterns, with the control roots differing from both salt-stressed and SA187 inoculated plants. Along PC2, the distinct responses of heat leaves and SA187 colonized heat leaves indicated that SA187 colonization significantly altered gene expression under heat stress. Salt-stressed samples such as salt leaves, salt roots, and SA187+ salt leaves clustered together, suggesting similar salt stress responses in leaves and roots, with SA187 inoculation under salt stress causing minor transcriptome changes. The control roots remained distinctly separate, highlighting the unique baseline gene expression in the roots compared to the stressed conditions.

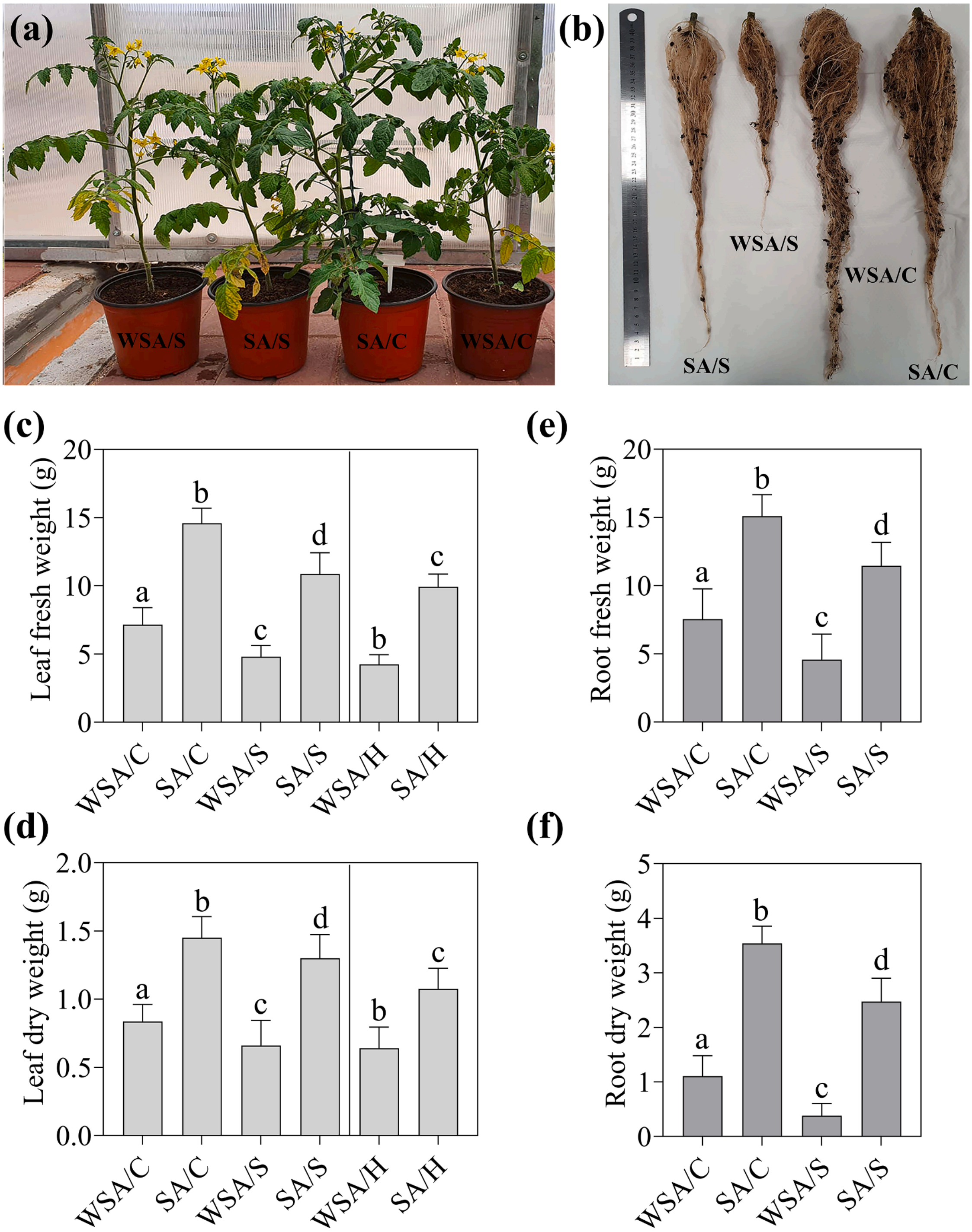

Fig. 1. Tomato plant growth after four weeks in a greenhouse under 200 mM NaCl stress. (a) Plant growth under salt stress treatment conditions. Plants colonized with SA187 under salt stress exhibited enhanced growth compared with salt-stressed plants without SA187. (b) Root morphology across salt stress treatments. (c) Leaf fresh weight of tomato plants. (d) Leaf dry weight of tomato plants. (e) Root fresh weight of tomato plants (f) Root dry weight of tomato plants. Bar graphs depicting tomato plants fresh and dry weight across different treatments displaying the mean values derived from three replicates. Different alphabets represent significant differences at $p < 0.05$, between the responses of the treatments given to the plants, and error bars indicate SD. WSA/C- Without SA187 under control condition, SA/C- SA187 inoculated under control condition, WSA/S- Without SA187 under salinity stress condition, SA/S- SA187 inoculated under salinity stress condition, WSA/H- Without SA187 under heat stress condition, SA/H- SA187 inoculated under heat stress condition.

3.4.2. *Regulation of differentially expressed genes (DEGs) in SA187 colonized tomato plants under salt and heat stress*

In leaves, the salt non-inoculated plants displayed the highest count of upregulated DEGs (947), followed by salt- SA187 inoculated plants (732), whereas the SA187 inoculated plants under control condition showed the lowest count (580) (Fig. 5a). Whereas, in roots the high number of upregulated DEGs were observed in the salt-SA187 inoculated plants (773), followed by salt non-inoculated (706), while the lowest number (450) was observed in SA187 inoculated plants under control condition (Fig. 5b). Contrary under heat stress, the count variation in upregulated genes in leaves does not show difference between SA187 inoculated and non-inoculated plants (Fig. 5c). The variation in

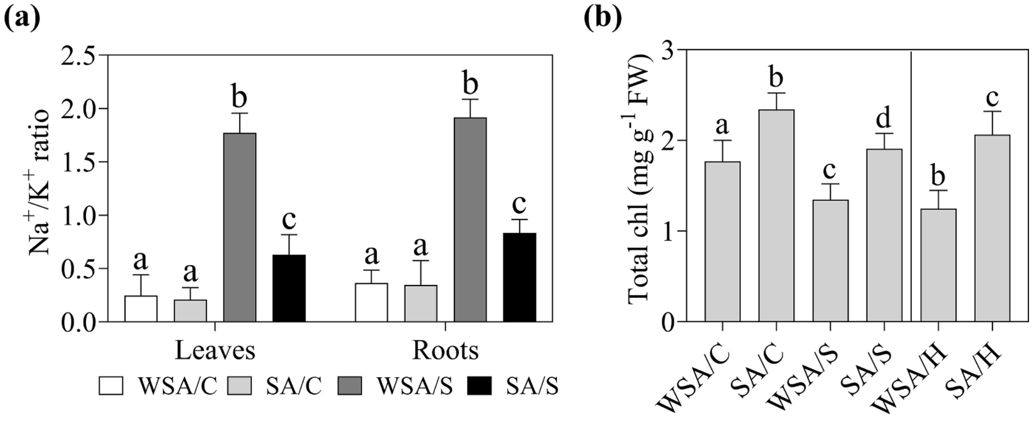

Fig. 2. Na^+/K^+ ratio and chlorophyll content of the tomato plants under the salt and heat stress condition. Bar graphs illustrating the means of three replicates of the (a) Na^+/K^+ ratio in tomato leaves and roots and (b) the total chlorophyll content. WSA/C- Without SA187 under control condition, SA/C- SA187 inoculated under control condition, WSA/S- Without SA187 under salinity stress condition, SA/S- SA187 inoculated under salinity stress condition, WSA/H- Without SA187 under heat stress condition, SA/H- SA187 inoculated under heat stress condition. Different letters display the significant difference at $p < 0.05$, between the treatment responses of the plants, and error bars indicate SD.

DEGs between the treatments showed to a certain extend how the colonization of SA187 modulated the tomato function responses under normal condition versus salinity or heat stress.

DEGs were identified in SA187-colonized and non-colonized tomato plants under salt and heat stress, revealing unique gene expression changes for each treatment. Comparative transcriptomic analysis showed that under control conditions, no DEGs were shared between SA187-inoculated and non-inoculated plants, indicating that SA187 inoculation had low influence on gene expression in the absence of stress.

In response to salinity stress, several key genes were differentially expressed, with distinguishable variations between the colonized and non-colonized plants. In leaves, genes such as *Solyc10g006080* (*NHX3*), a sodium/proton antiporter for ionic balance, and *Solyc05g052030* (*ERF4*), a transcription factor involved in ethylene-mediated stress responses, were significantly upregulated (Fig. 6). Additionally, genes expressed in both leaves and roots, including *Solyc12g009570* (*SOS2*) and *Solyc02g091340* (*SOS4*), essential components of the SOS pathway for ionic homeostasis, and *Solyc04g054730* (*SULTR3;5*), a sulfate transporter supporting sulfur assimilation under stress, showed enhanced activity. Under heat stress, fewer DEGs were observed compared to salinity stress, with most being heat shock proteins (HSPs) predominantly expressed in leaves (Fig. 6). Genes such as *Solyc03g123540* (*HSP17.4B*), *Solyc08g062450* (*HSP17.6*), *Solyc11g020330* (*HSP22.0*), and *Solyc12g042830* (*HSP26.5*) were overexpressed, indicating their role in thermotolerance by stabilizing proteins and reducing heat-induced damage.

3.5. qRT PCR of the differential gene expression under salt and heat stress

The RNA-seq data of SA187 inoculated and non-inoculated plants under the conditions of control and stress showed a large number of differentially expressed salt- and heat- stress responsive related genes including those involved for ion homeostasis and thermprotection of molecules in plants. Consistent with these data, the findings of qRT-PCR analysis confirmed the expression of the upregulated genes was involved in the plants tolerance against salinity and heat stress conditions.

3.5.1. SA187 colonization modulates salt stress-responsive genes

We investigated the molecular responses of salt-stressed tomato plants inoculated with the PGPB SA187, focusing on the expressions relative to stress-responsive genes involved in Na^+ and K^+ homeostasis, including *NHX3* (high-affinity potassium transporter 1), *SOS2* and *SOS4* (Salt Overly Sensitive 2 and 4), *LEA2* (Late Embryogenesis Abundant), and *ERF4* (Ethylene Responsive Factor 4). Real-time PCR was performed using the cDNA synthesized from the inoculated and non-inoculated tomato plants leaves and roots RNA under the control and salinity stress conditions. For the *NHX3* gene, no significant difference in expression was found between SA187-inoculated and non-inoculated plants under control conditions in the leaves (Fig. 7a). However, under salt stress, a significant difference was found with inoculated plants exhibiting a 4-fold increase in the expression level of *NHX3* gene in contrast to non-inoculated plants. In roots, *NHX3* expression showed a slight increase in inoculated plants under salinity stress, whereas non-colonized plants had a 2-fold higher expression under salinity stress contrast to the colonized plants (Fig. 7a). The expression of the SOS2 gene did not vary significantly under control conditions in the leaves, but a 3-fold increase was observed in SA187-inoculated plants under salinity stress indicating a significant difference (Fig. 7b). In roots, *SOS2* expression showed no significant difference between the inoculated and non-inoculated plants. However, it significantly differed under salt stress condition, with a 2-fold increase in the colonized to non-colonized plant roots under salinity stress (Fig. 7b). Similarly, *SOS4* expression was comparable in roots of inoculated and non-inoculated plants under control conditions but showed a slight increase in both leaves and roots of SA187-inoculated plants under salinity stress (Fig. 7c).

For the *LEA2* gene, no significant difference was identified between inoculated and non-inoculated plants under control conditions (Fig. 7d). However, under salinity, non-inoculated plants exhibited higher expression levels in leaves, while no significant difference was found in roots under either treatment conditions. Furthermore, the expression of *ERF4* did not vary significantly between treatments under control conditions in leaves. However, a significant difference was observed under salinity stress condition, indicating a slight increase in expression of inoculated plant leaves (Fig. 7e). In contrast, no changes were observed in inoculated and non-inoculated plants roots under normal and salinity stress condition. For the *SULTR3;5* gene, no significant difference was observed in leaves under control conditions. Whereas under salinity stress, SA187-inoculated plants displayed a 2.5-fold increase in *SULTR3;5* expression in leaves and a 3-fold increase in roots compared to non-inoculated plants (Fig. 7f).

3.5.2. SA187 colonization modulates heat stress-responsive genes

Under heat stress, the relative expression levels of various stress-responsive genes in the leaves of SA187-colonized and non-colonized tomato plants showed varied differences. For the *LEA2* gene, no significant difference was observed under control conditions between inoculated and non-inoculated plants (Fig. 8a). However, under heat stress, a

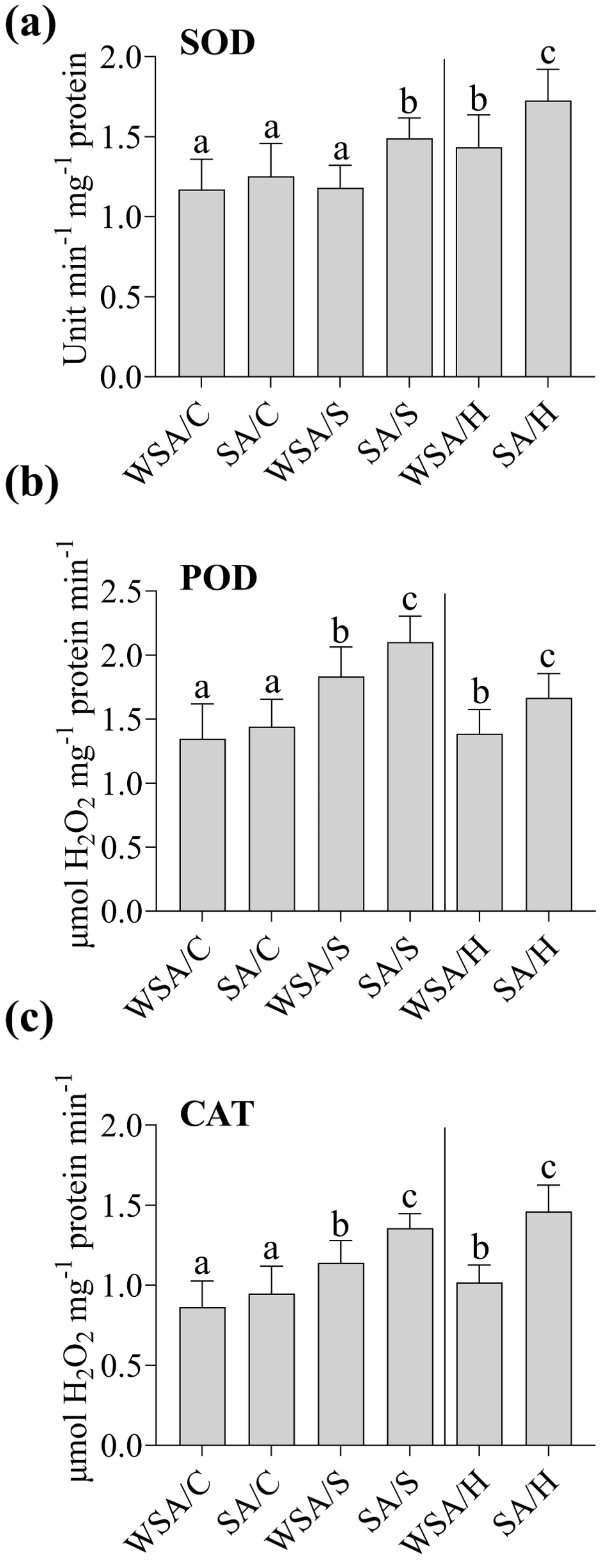

Fig. 3. Antioxidant activities of tomato plants under salt and heat stress condition. Bar graphs illustrate (a) Leaf SOD activity, (b) Leaf POD activity, and (c) Leaf CAT activity of tomato plants exposed to different treatments. WSA/C-Without SA187 under control condition, SA/C- SA187 inoculated under control condition, WSA/S- Without SA187 under salinity stress condition, SA/S- SA187 inoculated under salinity stress condition, WSA/H- Without SA187 under heat stress condition, SA/H- SA187 inoculated under heat stress condition. Different letters display significant differences at $p < 0.05$, between the responses of plants, and error bars indicate SD.

significant difference was found, with non-inoculated plants exhibiting higher *LEA2* expression than SA187-colonized plants. Similarly, for the *AUX* gene, no significant difference was identified in the inoculated and non-inoculated plants leaf under control conditions. Under heat stress, a significant difference emerged, with higher *AUX* expression in non-inoculated plants (Fig. 8b).

For the *HSP17.4B* gene, no significant difference was observed in leaves under control condition. However, under heat stress, a significant difference was observed, with a moderate increase in expression in the leaves of SA187-colonized plants (Fig. 8c). In addition, the *HSP17.6* gene displayed no significant difference in leaves under control conditions, but under heat stress, colonized plants showed an appropriate increase in expression compared to non-colonized plants (Fig. 8d).

Furthermore, the *HSP22.0* gene showed no significant differences in expression in leaves under control conditions. However, under heat stress, there was a significant difference, with SA187-colonized plants exhibiting a slight increase in expression in leaves (Fig. 8e). For the *HSP26.5* gene, the expression was similar between colonized and non-colonized leaves under control conditions but showed a significant difference under heat stress, with a slight increase in expression of SA187-colonized plants (Fig. 8f). Our results show specific and gene-dependent effects of SA187 colonization on stress-responsive gene expression under heat stress, particularly in leaf tissues.

4. Discussion

The beneficial bacterial species use several strategies to support plant growth under diverse environmental conditions. SA187 has been shown to colonize the roots of various plant species, and a certain level of host specificity has been observed in Arabidopsis and alfalfa (Synek et al., 2021). Previous research has reported that endophytic root colonization by SA187 was absent in tomato, while epiphytic colonization of SA187 was observed (Synek et al., 2021). This specificity may be attributed to two factors, the varying compatibility between SA187′s enzymatic arsenal, the unique cell wall composition of different plants, and the distinct patterns of root exudation among various plant species or taxonomic groups (Popper, 2008). Therefore, PGPB SA187 as an epiphyte, to investigate its beneficial role in abiotic stress tolerance of tomato plants.

The interacting mechanisms are influenced not only by the microbes involved but also by the plant species, ecotype, developmental and physiological state, plant immunity, and the surrounding abiotic or biotic environment (Tiwari et al., 2024; Ahmad et al., 2024; Shamim et al., 2024). We found that colonization with SA187 significantly enhanced tomato growth under both saline and heat stress conditions, outperforming the non-inoculated plants. SA187 colonization induced morphological, physiological, and molecular changes, including longer and broader root growth, increased fresh and dry weights, and improved the chlorophyll content and antioxidant activity. This positive interaction has been observed in diverse plants, including Arabidopsis (Kim et al., 2014), rice (Khan et al., 2020), and maize (Nadeem et al., 2009). It has also been shown that specific bacteria colonizes roots for abiotic stress tolerance (Abdul Aziz et al., 2024).

The accumulation of Na^+ ions has detrimental effects on plants, causing early aging, reduced photosynthetic efficiency, and disrupted metabolic functions. Furthermore, Na^+ interferes with K^+ for both membrane transport and enzymatic activity, thereby hindering plant development (Abdul Aziz and Masmoudi, 2023a). Most plant cells have evolved strategies to mitigate the harmful effects of Na^+ buildup by maintaining K^+ levels and actively expelling Na^+ from roots (Assaha et al., 2017; Khader et al., 2024). In this study, plants colonized with SA187 exhibited lower concentrations of Na^+ than K^+ ions in both leaves and roots. Consequently, the decreased Na^+/K^+ ratio observed in SA187-colonized plants under saline conditions demonstrates SA187's ability to enhance plant tolerance to salt stress. Previous research on tomato seedlings with endophytic halotolerant *Bacillus velezensis* FMH2

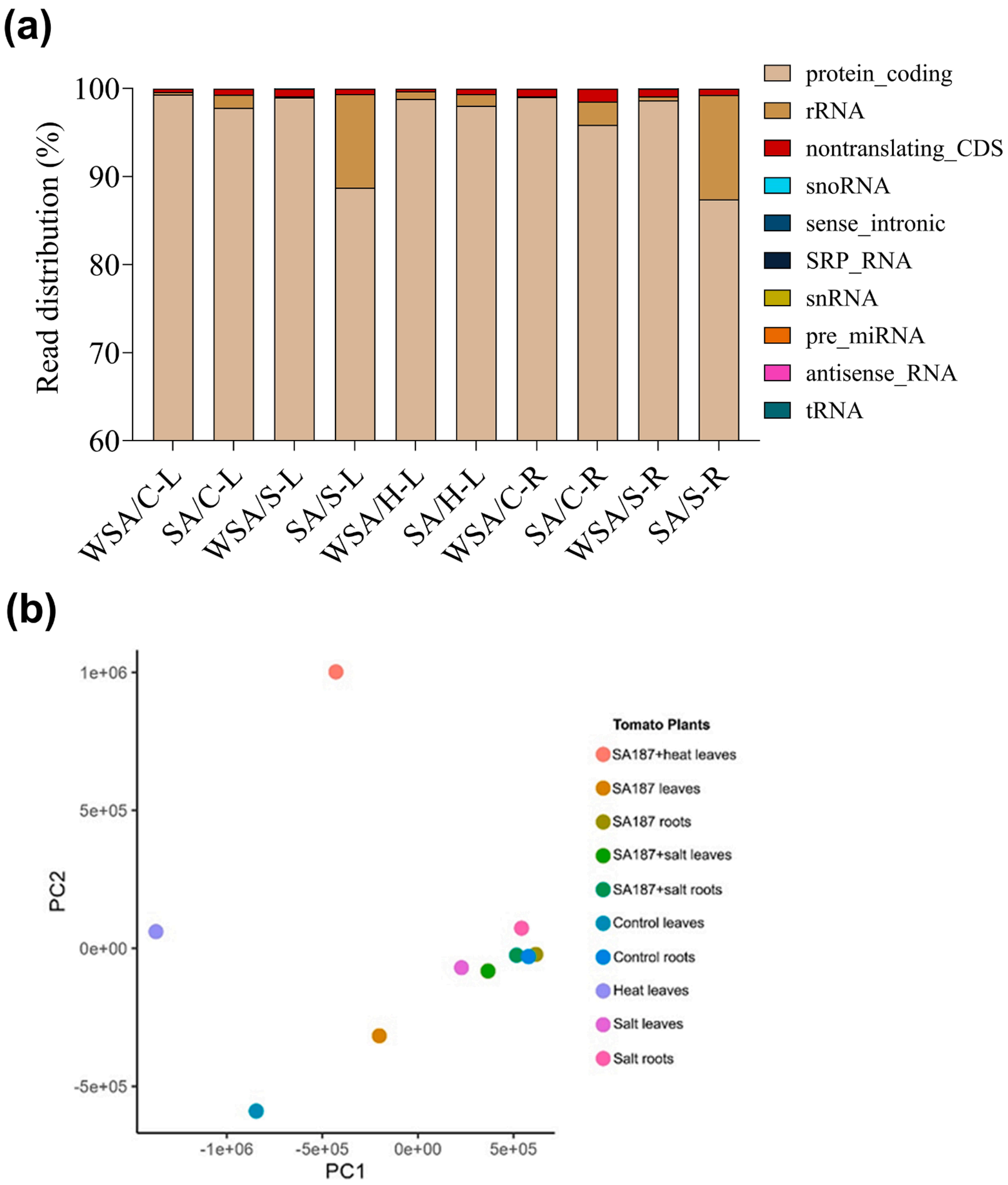

Fig. 4. RNAseq analysis of tomato plant leaves and roots under salt and heat stress condition. (a) Total read distribution of tomato plant RNAseq. (b) PCA plot illustrating the clustering of samples based on different treatments. The first two principal components, PC1 and PC2, elucidated the variation in the data, indicating the impact of treatments on the overall biological response in tomato plants. WSA/C-L- Without SA187 under control condition leaves, SA/C-L- SA187 inoculated under control condition leaves, WSA/S-L- Without SA187 under salinity stress condition leaves, SA/S-L SA187 inoculated under salinity stress condition leaves, WSA/H-L- Without SA187 under heat stress condition leaves, SA/H-L SA187 inoculated under heat stress condition leaves, WSA/C-R- Without SA187 under control condition roots, SA/C-R- SA187 inoculated under control condition roots, WSA/S-R- Without SA187 under salinity stress condition roots, SA/S-R SA187 inoculated under salinity stress condition roots.

mitigated salt stress in tomato plants by regulating ion accumulation (reducing internal Na+ buildup, increasing K^+ and Ca^{2+} absorption) and enhancing antioxidant responses (Masmoudi et al., 2021). Inoculating roots with *Azotobacter chroococcum* 76A not only enhanced tomato adaptation to salt stress in low-nitrogen environments, but also improved nutrient assimilation efficiency under moderate and severe salinity, demonstrating its potential for enhanced nutrition and salt stress protection (Van Oosten et al., 2018). Various genomics have indicated different mechanisms for studying plant abiotic stress tolerance (Abdul Aziz et al., 2022). Comparative transcriptomic and metabolomic studies have indicated that *Pseudomonas oryzihabitans* AXSa06 promotes salt tolerance in tomatoes by effectively activating antioxidant metabolism, reducing stress signals, detoxifying Na^+, and efficiently assimilating carbon and nitrogen (Mellidou et al., 2021).

Antioxidant enzymes can reduce the toxic effects of ROS in plants (Zandi and Schnug, 2022; Abdul Aziz and Masmoudi, 2024). Analysis of antioxidant enzymes in salt- and heat-stressed tomato plants demonstrated that SA187 enhanced plant stress tolerance by promoting more robust antioxidant defenses, thereby mitigating the impact of oxidative damage caused by stress. To protect the plant from stress, SA187 facilitated higher antioxidant defense mechanisms, suggesting that salt stress induced more oxidative stress, and bacterial treatment had a more pronounced effect under saline conditions. This observation confirms that abiotic stress and SA187 both stimulate antioxidant defense mechanisms in plants, with SA187 contributing to an increase in the antioxidant activities. A recent study on tomato plants using halotolerant PGPR strain showed improvements in dry biomass, growth, and antioxidant levels (Latif et al., 2024). Other studies have reported

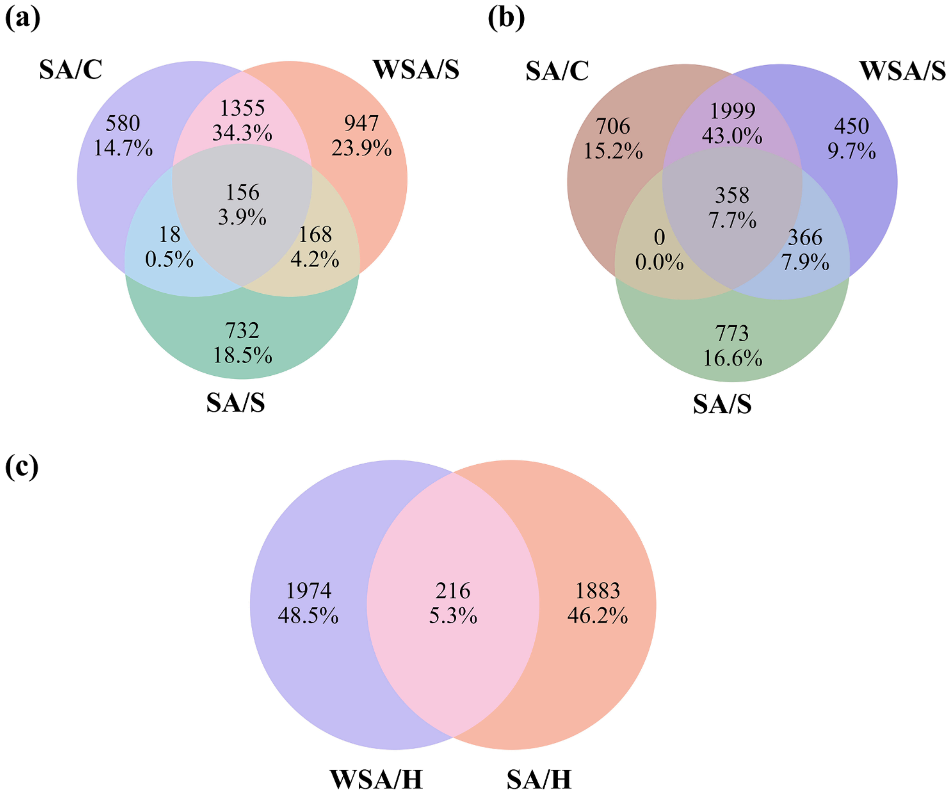

Fig. 5. Illustration of the gene upregulation in SA187 inoculated and non-inoculated plants under the conditions of salt and heat stress. Venn diagram of the upregulated genes in (a) leaves of tomato plants under salinity stress. (b) roots of tomato plants under salinity stress. (c) leaves of tomato plants under heat stress. SA/C- SA187 inoculated plants under control condition, WSA/S- without SA187 under salt stress, SA/S- SA187 inoculated plants under salt stress, WSA/H- without SA187 under heat stress, and SA/H- SA187 inoculated plants under salt stress.

increased antioxidant enzyme activity in plants inoculated with PGPR under saline conditions (Saberi-Riseh et al., 2020; Yasmeen et al., 2020; Zuluaga et al., 2021). In a study by Azeem et al. (2022), maize plants treated with the halotolerant bacteria *Bacillus safensis* PM22 displayed higher SOD and CAT activity when exposed to salinity stress (Azeem et al., 2022).

The transcriptome analysis of tomato leaves and roots was performed to examine the interaction of SA187 with tomato plants at the molecular level. RNA-seq revealed alterations in gene expression within the leaves and roots of tomato plants colonized by SA187, enhancing their tolerance to salt and heat stress. The DEGs analysis showed that SA187 inoculation improves the plant's ability to manage ionic balance and sulfate metabolism under salinity stress and thermotolerance genes were highly expressed under the heat stress condition. This shows the unique molecular pathways activated by plants to cope with different stress conditions, with SA187 playing a significant role in modulating stress-specific gene expression to enhance the tolerance. The observed changes in gene expression patterns between colonized and non-colonized tomato plants under salt and heat stress were evaluated through qRT PCR.

The current study demonstrated that Enterobacter sp. SA187 enhances plant growth under saline conditions by upregulating the expression of salt-responsive transporters, including *NHX3, SOS2, SOS4, ERF4*, and *SULTR3;5*, which regulates ion homeostasis in tomatoes (Latif et al., 2024). Four NHX antiporters have been identified in tomatoes, with SlNHX4 and SlNHX3 showing significant up-regulation under saline conditions (Gálvez et al., 2012; Gharsallah et al., 2016). Similarly, it was clear from the current study that the expression of *NHX3* in the leaves of tomato plants colonized with SA187 played an important role in ion homeostasis. Our results corroborate those of a previous study conducted on maize plants under salinity stress, in which *Bacillus amyloliquefaciens* inoculation enhanced *NHX3* gene expression. In addition, Latif et al. (2024) reported that *NHX3* gene expression was upregulated when a group of halotolerant PGPR colonized tomato plants under salt stress.

The salt-overly sensitive (SOS) signaling pathway genes play a crucial role in preserving ions homeostasis in plants under salinity stress. *SOS2* regulates tonoplast Na^+ exchange, and its overexpression improve salt tolerance in tomatoes (Huertas et al., 2012), whereas *SOS4* gene may be involved in the regulation of Na^+ and K^+ homeostasis (Shi et al., 2002). A subcellular localization prediction showed that SOS2 and SOS4 proteins in tomatoes were located on the cytoplasm and chloroplast (Huang et al., 2024). In the present study, *SOS2* and *SOS4* genes were upregulated in the leaves and root tissues when plants were exposed to salt stress, implying their role in leaf- and root- specific responses. Previous reports have shown that PGPR-inoculated plants have higher *SOS* gene expression under salt stress than uninoculated plants, suggesting their participation in salt tolerance mechanisms (Bharti et al., 2016). It was found that *Paenibacillus yonginensis* inoculation enhanced *SOS2* gene upregulation in *Panax ginseng* under salt stress (Sukweenadhi et al., 2018). In addition, increased *S0S2* expression was observed in salt stressed Arabidopsis plants inoculated with *Pseudomonas knackmussii* (Rabhi et al., 2018). Thus, the improved *SOS2* and *SOS4* gene expression in SA187 inoculated tomato roots can be associated with an enhancement in the plant growth under the salinity stress.

Many crops contain late embryogenesis abundant (LEA) proteins, which are involved in adaptation to various environmental stresses. They are present and expressed in plants of the extreme environmental conditions (Abdul Aziz et al., 2023). These proteins play a crucial role in helping plants cope with adverse conditions such as drought, high salinity, and extreme temperatures (Abdul Aziz et al., 2021). LEA proteins are crucial for stress tolerance in plants as they protect cells from

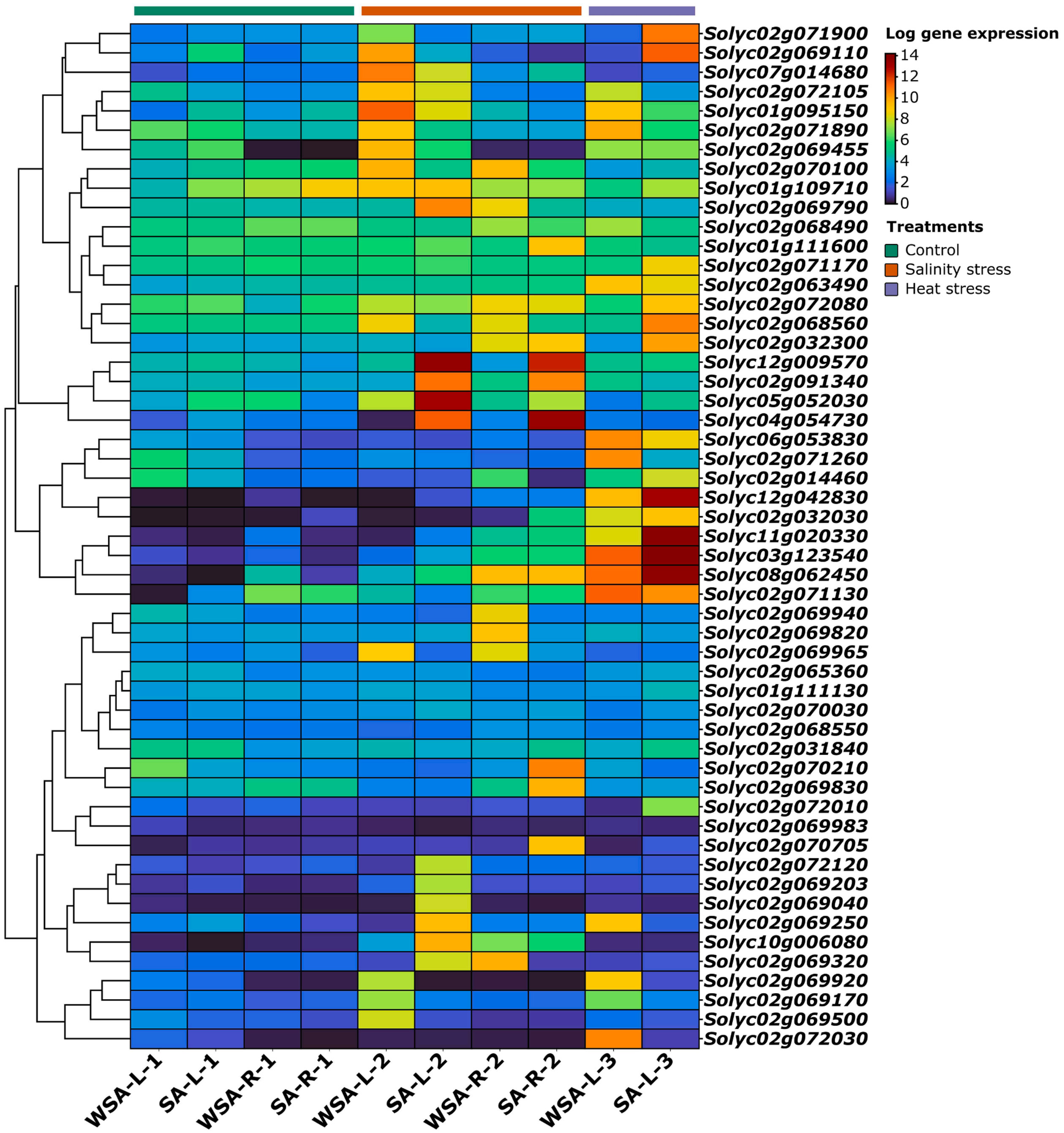

Fig. 6. Heatmap of DEGs in SA187-inoculated and non-inoculated tomato plants under the conditions of control, salinity stress, and heat stress. The color gradient represents the log-transformed gene expression levels, with dark blue showing low expression and red displaying high levels of gene expression. The hierarchical clustering on the left assembles genes based on expression similarity across the treatments. WSA-L- without SA187 leaves, SA-L- with SA187 leaves, WSA-R- without SA187 roots, and SA-R- with SA187 roots.

extreme conditions by stabilizing proteins and cellular structures (Abdul Aziz and Masmoudi, 2023b). Salt-treated tomato leaves showed the most significant increase in LEA expression levels, suggesting that salt stress strongly induces *LEA* gene expression in leaves. However, SA187 colonization under salt stress and heat stress showed no upregulation of *LEA2* gene expression in the leaves and roots. Certain *LEA* genes are upregulated upon bacterial colonization, although this has been less extensively studied. For instance, LEA proteins have been linked to increased tolerance and activation of plant defense mechanisms against bacterial colonization as reported in Arabidopsis, maize and tobacco (Hanin et al., 2011; Liu et al., 2013; Nagaraju et al., 2019). Furthermore, it was found that heat stress with SA187 resulted in a moderate increase in *LEA2* expression level lower than non-inoculated plants expression level under heat stress, implying that the stress condition is a major trigger for *LEA2* gene expression with or without inoculation. The root tissues had minimal effect on *LEA2* expression under the heat and salt stress. Previous studies on LEA proteins have shown that these genes play major roles in plants subjected to high salinity and heat stress (Cao and Li, 2015; Jia et al., 2022; Sabeem et al., 2022). However, upon SA187 colonization, the expression of LEA gene was minimal in both salt- and heat-stressed tomato plants.

The ERF4 is part of the AP2/ERF (APETALA2/ethylene-responsive element-binding factor) family of transcription factors. They play a significant role in plant stress responses, particularly under abiotic stress

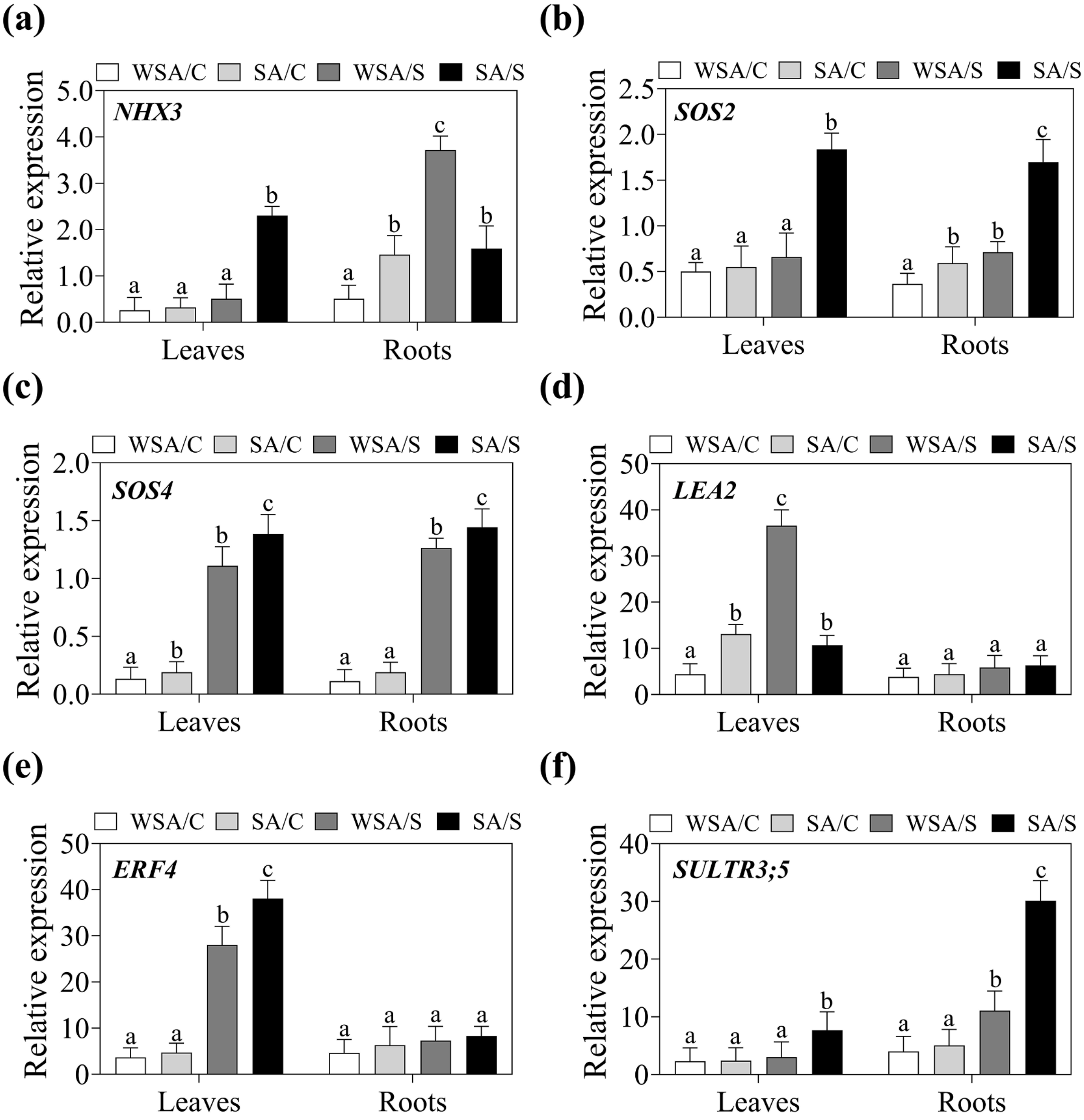

Fig. 7. qPCR analysis of stress-responsive gene expression in folds for SA187 inoculated and non-inoculated plants under salt stress. (a) Expression level of NHX3 gene. (b) Expression level of SOS2 gene. (c) Expression level of SOS4 gene. (d) Expression level of LEA2 gene. (e) Expression level of ERF4 gene. (f) Expression level of SULT3;5 gene. WSA/C- Without SA187 under control condition, SA/C- SA187 inoculated under control condition, WSA/S- Without SA187 under salinity stress condition, SA/S- SA187 inoculated under salinity stress condition. The values shown by the different letters are significantly different at P value of 0.05.

(Pandey et al., 2005). In present study, leaf tissues showed varied ERF4 expression patterns under salt stress condition. Leaves of the plants inoculated with SA187 under salinity stress displayed an upregulation of *ERF4* gene expression in comparison to non-inoculated plants under salt stress. The *ERF4* gene exhibited minimal or no expression across all root treatments, indicating that SA187 inoculation or the salinity stress does not result in the upregulation of *ERF4* gene expression in the root tissues. Seo et al. (2010) reported that the overexpression of the *Brassica rapa* *ERF4* gene increased the Arabidopsis plants tolerance to salt stress.

SULTRs emerge as potential hub genes encoded by a distinct group of sulfate transporter genes. SULTR proteins play significant roles in various plant stress responses, including those involved in heavy metals, low temperatures, drought, salinity, and other abiotic factors (Huang et al., 2018; Yuan et al., 2021). In the roots, sulfur is taken up as sulfate, which is mainly governed by SULTR transporters. We found that SA187 increased sulfur levels in salt-stressed tomato roots. This phenomenon was previously observed in Arabidopsis roots inoculated with SA187 (Andrés-Barrao et al., 2021), indicating that sulfur metabolism plays an important role in salt stress tolerance. The *SULTR3;5* gene exhibited a strong response in roots, especially in SA187 colonized salt roots, compared with control roots, demonstrating an exceptionally high fold change. This suggests that *SULTR3;5* is essential for sulfate transport in the roots of SA187 colonized plants. In summary, bacterial and salt-related treatments strongly induced *SULTR3;5* transporter expression in the root tissues. This suggests that these sulfate transporter genes play distinct roles in the salt stress response mechanisms of tomato plants.

Heat stress-induced thermomorphogenesis relies heavily on the plant hormone auxin, and elevated temperatures affect both auxin movement and signaling pathways (Zhang et al., 2023). Under heat stress conditions, auxin-responsive proteins are vital for controlling various physiological and developmental functions of plants, enabling their adaptation and survival in harsh environments. These proteins significantly contribute to plant resilience by modulating gene expression,

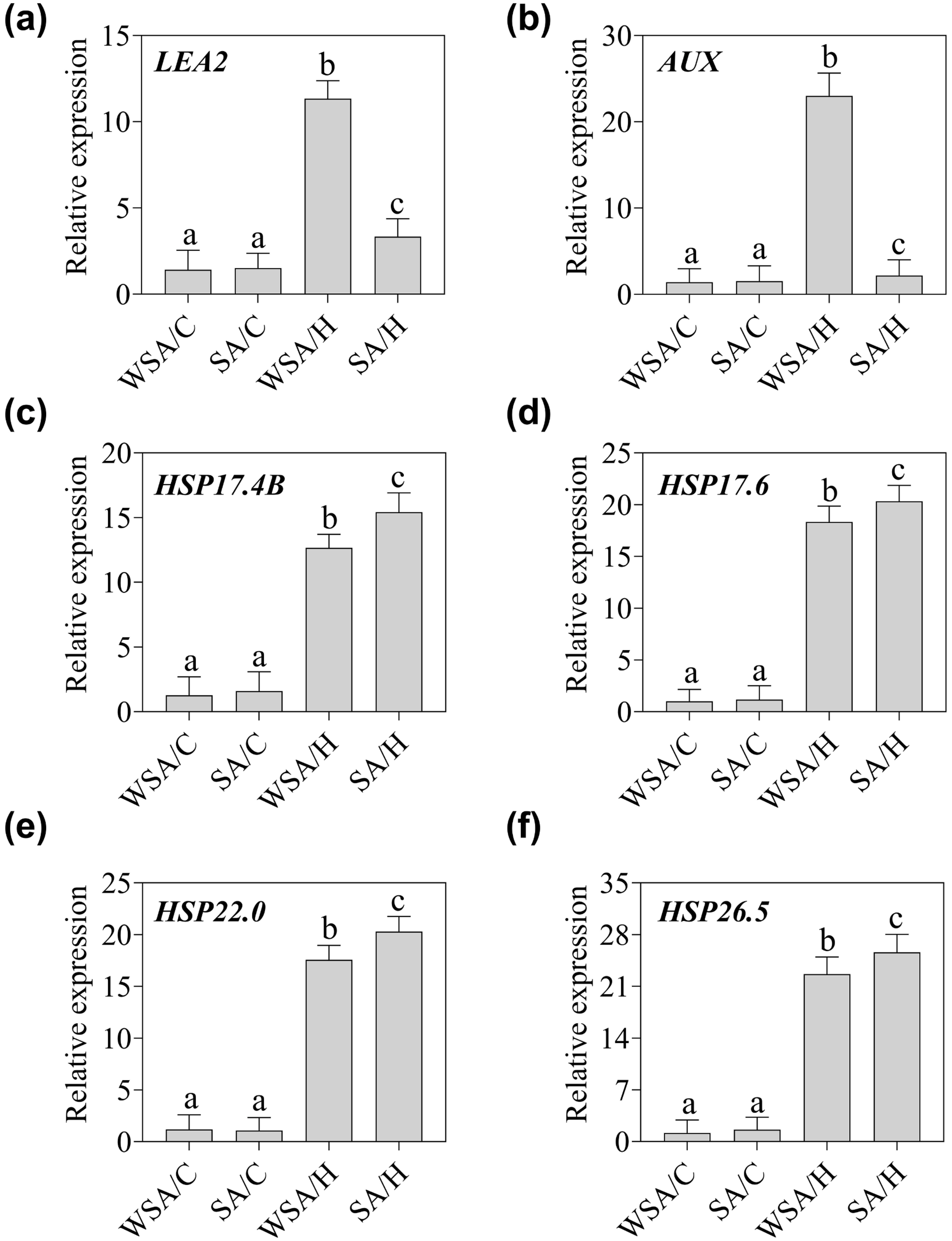

Fig. 8. qPCR analysis of stress-responsive gene expression in folds for SA187 inoculated and non-inoculated plants under heat stress in leaves. (a) Expression level of LEA2 gene. (b) Expression level of AUX gene. (c) Expression level of HSP17.4B gene. (d) Expression level of HSP17.6 gene. (e) Expression level of HSP22.0 gene. (f) Expression level of HSP26.5 gene. WSA/C- Without SA187 under control condition, SA/C- SA187 inoculated under control condition, WSA/H- Without SA187 under heat stress condition, SA/S- SA187 inoculated under heat stress condition. The values shown by the different letters are significantly different at P value of 0.05.

facilitating hormone interactions, and enhancing stress-tolerance mechanisms (Liu et al., 2023; Zhang and Zhou, 2023; Chen et al., 2024). In the present study, heat treatment resulted in the highest expression of the auxin-responsive proteins. However, SA187-colonized plants showed no major effect on the expression of the *AUX* gene. These findings indicate that, although heat stress typically causes a robust response involving auxin-responsive proteins, the interaction between plants and colonizing bacteria alters this response. This could mean that SA187 might provide alternative pathways for plants to cope with heat stress, potentially reducing their reliance on auxin-responsive proteins or indicating a form of adaptation. In summary, heat stress generally boosts auxin-responsive protein expression; however, bacterial colonization appears to reduce this specific response, implying a complex interaction between plant stress responses and microbial effects.

Under heat stress conditions, we observed some heat shock protein encoding genes *HSP17.4B, HSP17.6, HSP22.0,* and *HSP26.5* were induced slightly in contrast to their upregulation under heat stress with no inoculation. This indicates that the expression of *HSP* genes during heat stress were slightly modulated by the SA187, that exhibited an increased expression under the heat stress condition of tomato plants, indicating their important role in protecting plants from heat-induced damage. This is in relation to the previous work carried out on Arabidopsis showing that SA187 enhanced the expression of heat-responsive and memory genes upon heat stress (Shekhawat et al., 2021). Furthermore, it has been reported that *HSP22*.0 and *HSP26.5* enhanced heat stress tolerance of Arabidopsis and wheat by binding to the promoters of heat shock element (HSE) that regulate their heat tolerance (Wang et al., 2016; Bi et al., 2020). It has been revealed that HSPs could act as plant thermoprotectants, and their overexpression enhances thermotolerance (Bourgine and Guihur, 2021).

5. Conclusion

This study provides evidence that Enterobacter sp. SA187 significantly enhances tomato plant tolerance to abiotic stresses, particularly salinity and heat. Through a combination of physiological, biochemical, and molecular analyses, we demonstrated that SA187 inoculation helps to maintain ion homeostasis, enhances antioxidant enzyme activity, and upregulates key stress-responsive genes, improving tomato plant tolerance under adverse conditions. The novelty aspect of this study lies in elucidating the various role of SA187 in modulating stress adaptation mechanisms, indicating its potential as a promising PGPB for sustainable agriculture. Unlike conventional approaches, our findings show the ability of SA187 to simultaneously mitigate multiple stress factors, allowing for the innovative microbial-based stress management strategies. While these results are promising, further research is required to validate the long-term efficacy of SA187 under field conditions and assess its impact on crop yield and quality. Additionally, studying interactions between SA187 and other beneficial microbes could enhance its applicability in diverse agricultural settings. Our study enhances the understanding of plant-microbe interactions in stress tolerance and contributes to the microbiome-driven solutions for improving crop tolerance in a period of escalating environmental challenges.

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CRediT authorship contribution statement

Shafeeq Rahman: Writing – original draft, Methodology, Investigation, Formal analysis. **Aysha Ali Rahmani:** Writing – original draft, Methodology, Investigation, Formal analysis. **Mughair Abdul Aziz:** Writing – review & editing, Visualization, Formal analysis. **Manzoor Ahmad:** Investigation, Formal analysis. **Sathesh K. Sivasankaran:** Validation, Formal analysis, Data curation. **Heribert Hirt:** Writing – review & editing. **Khaled Masmoudi:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships and have no conflicts of interest.

Data availability

The datasets generated and analysed during the current study are available in the NCBI repository as Sequence Read Archive (SRA) data under BioSample accessions SAMN43765222, SAMN43765223, SAMN43765224, SAMN43765225, SAMN43765226, SAMN43765227, SAMN43765228, SAMN43765229, SAMN43765230, SAMN43765231.

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