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Research Article

The Effect of *Bacillus Cereus* on the Ion Homeostasis, Growth Parameters, and the Expression of Some Genes of Artemisinin Biosynthesis Pathway in *Artemisia Absinthium* Under Salinity Stress

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Background: Soil salinity is a major problem in the world that affects the growth and yield of plants. Application of new and up-to-date techniques can help plants in dealing with salinity stress. One of the approaches for reducing environmental stress is the use of rhizosphere bacteria.

Objective: The aim of present study was to investigate the effect of the inoculation of Bacillus cereus on physiological and biochemical indicators and the expression of some key genes involved in the Artemisinin biosynthesis pathway in Artemisia absinthium under salinity stress.

Materials and Methods: The study was conducted using three different salinity levels (0, 75, 150 mM/NaCl) and two different bacterial treatments (i. e, without bacterial inoculation and co-inoculation with B. cereus isolates). The data from the experiments were analyzed using factorial analysis, and the resulting interaction effects were subsequently examined and discussed.

Results: The results showed that with increasing salinity, root and stem length, root and stem weight, root and stem dry weight, and potassium content were decreased, although the content of sodium was increased. Rhizosphere bacteria increased the contents of Artemisinin, potassium, calcium, magnesium, and iron and the expression of Amorpha-4,11-diene synthase and Cytochrome P450 monooxygenase1 genes as well as the growth indicators; although decreased the sodium content. The highest ADS expression was related to co-inoculation with B. cereus isolates E and B in 150 mM salinity. The highest CYP71AV1 expression was related to co-inoculation with B. cereus isolates E and B in 150 mM salinity.

Conclusion: These findings showed that the increase in growth indices under salinity stress was probably due to the improvement of nutrient absorption conditions as a result of ion homeostasis, sodium ion reduction and Artemisinin production conditions by rhizosphere B. cereus isolates E and B.

Keywords: Artemisia absinthium, Amorpha-4,11-diene synthase, Cytochrome P450 monooxygenase1, Rhizosphere bacteria

1. Background

Salinity is one of the most important factors that affects the physiological processes in plants (1). The effects of salinity stress are the results of complex interactions between morphological, physiological, and biochemical processes. Salinity stress has a negative effect on

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germination and growth as well as physiological processes (photosynthesis, respiration and sweating), nutrient balance, membrane and cellular homeostasis characteristics, and enzymatic and metabolic activities (2, 3). A decrease in soil osmotic potential (osmotic stress) and an increase in ion toxicity (tension) are from the common consequences of salinity stress that plants experience. Salinity also creates secondary stresses such as oxidative stress (production of reactive oxygen species, ROS and nutritional and hormonal imbalance for plants (4). Plants develop various mechanisms such as activation of antioxidant enzymes or synthesis of non-enzymatic antioxidant compounds, ion homeo-stasis, polyamine accumulation, biosynthesis of com-patible solutions or osmotic protectors, nitric oxide production, absorption and transport of ions, and hormone modulation for salinity tolerance (5-7). Plant growth promoting rhizobacteria (PGPR) are beneficial soil microorganisms that can be used as natural resources in agriculture to improve soil quality and plant growth and yield (8). PGPR can directly and indirectly increase plant growth. Among the methods applied for promoting plant growth by bacteria is the dissolution of phosphate in a bioavailable form through mineralization processes. Another method of promoting plant growth is the production of auxins. Indole-3-acetic acid (IAA) is the main member of the auxin family which is produced by various bacteria, fungi and algae. On the other hand, by increasing the number of root branches, capillary and lateral roots; the length of the plant root, and as a result the absorption of nutrients around the root increases (9-12). PGPR plays an important role in plant tolerance against abiotic and biotic stresses and improving the relationship between plants and microbes. Studies have shown that PGPR induces tolerance to various abiotic stresses with physiological changes in plants under salinity, drought, and heavy metal stresses leading to the improvement of plant growth in these conditions. In addition, PGPR stimulates the tolerance system by increasing the antioxidant activities in the plant through the production of relative enzymes and the accumulation of effective metabolites which regulate plant growth by several mechanisms. Secondary metabolites, antibiotics, and volatile compounds produced in these conditions increase the plant's resistance to salinity stress and counteract the destructive effects of the stress (13). *Bacillus* species are Gram-positive, rodshaped bacteria and a member of the Firmicutes order which can act as rhizosphere bacteria. The production of antimicrobial substances and sporulation capacity provides a double advantage for the survival of *Bacillus* spp. in different habitats. Therefore, these bacteria can be seen in different environments such as soil, plant rhizosphere, and sea water. Despite the previous studies on potential of *Bacillus* spp. for use in plant rhizosphere (14). *A. absinthium L.*, in *Asteraceae* is a perennial woody plant that grows widely in dry and sunny areas of Eurasia, North Africa, North and South America. This plant may be recognized by its silvery gray leaves with a soft silky texture (15). A compound called Artemisinin, in *A. absinthium* (16), is a sesquiterpene lactone (17). Artemisinin has strong anticancer (18) and antimalarial (19) properties. It has also been observed that Artemisinin prevents the penetration of viruses into the cells and proliferation of them in the cells both in the body and in cell cultures (20). One of the key genes in the Artemisinin biosynthesis pathway is *ADS*, which codes for Amorpha-4,11-diene synthase; a key enzyme in the Artemisinin biosynthesis pathway (21). Another key enzyme in the path of Artemisinin biosynthesis is (*CYP71AV1*) (22). *A. absinthium* is one of the medicinal plants with valuable medicinal properties that can survive in saline conditions to some extent. New techniques can be used to improve the reduction of the effects of salinity stress on this plant. One of these new techniques is the use of rhizosphere bacteria. The effect of this rhizosphere bacteria on *A. absinthium* has not been yet investigated.

2. Objective

In this research, the effect of rhizosphere bacteria on the absorption of sodium and potassium ions and Artemi-sinin production, as well as the expression of genes (ADS and CYP71AV1) was studied in Artemisinin biosynthesis pathway in A. absinthium under salt stress.

3. Materials and Methods

3.1. Isolation, Identification, and Selection of Rhizosphere Bacteria

Bacteria were isolated from the rhizosphere of *A. absinthium* on nutrient agar culture medium (Merck, Germany; included 0.5% peptone, 0.3% meat extract, 0.3% yeast extract, 0.5% NaCl, and 1.5% agar) and

following the purification of bacterial colonies, the isolates were screened by the measurement of the production of auxin and phosphate (23). PCR test was performed for molecular identification of selected isolates through the amplification of 16S rRNA gene by universal primers (**Table 1**) and then determining the nucleotide sequence of PCR products. The PCR reaction protocol included an initial denaturation at 94 ºC for 5 min followed by 35 cycles of denaturation at 94 ºC for 30 s, primers annealing at 55 ºC for 30 s, and extension at 72 ºC for 1 min. Each reaction was ended by a final extension at 72 ºC for 10 min. In the last step, the ability of selected bacteria to grow in salt with different concentrations was checked. After the end of the tests, the concentration of 10^9 $CFU.mL^{-1}$ of the selected bacteria was prepared to inoculate the soil (24).

3.2. Planting and Treatments of A. Absinthium

Seeds were purchased from Pakan Seed Company (Iran) and were cultivated in greenhouse conditions in sterile soil containing peat moss, coco peat, perlite with proportions of 50%, 30%, and 20%, respectively. After the plants reached the multi-leaf stage, the soil around the plant in the root zone was inoculated with a concentration of 10^9 $CFU.mL^{-1}$ in two levels (without bacterial inoculation and co-inoculation with the isolated bacilli). After two weeks, the plants under saline treatment with three levels salinity (0, 75, and 150 mM NaCl) was applied with three repetitions. Plant leaf samples were taken after 72 hours to investigate gene expression and after 21 days to investigate other indicators (25).

3.3. Measurement of Artemisinin Composition by High Performance Liquid Chromatography (HPLC) Method

For this purpose, first, 1 g of leaf sample was ultrasonicated with 20 mL of methanol for 30 min, and in the next step, it was filtered and then 20 µL of the resulting extract was injected into the HPLC apparatus (Knauer, Germany). Also, in order to quantitatively analyze the composition of Artemisinin, the relevant calibration curve was drawn by injecting standard *Artemisinin* 98% (Sigma-Aldrich, 361593) with certain concentrations to the apparatus and obtaining the area under the peak of each. Then by using the linear equation of this calibration curve, the total amount of Artemisinin was determined in terms of mg per gram of the plant dry weight. HPLC apparatus used in this study was a KNAUER model, which featured a UV detector operating at a specified wavelength (nm) and using a C18 column with a length of 15 cm, a diameter of 4.6 mm and a flow rate of 0.3 ml/min at the wavelengths of 250 to 300 nm (26,27).

3.4. Ion Measurement by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

To measure the amount of sodium, calcium, magnesium, potassium, and iron ions, first, 4 mL of concentrated HNO_3 and 1 mL of 30% H_2O_2 were added to 0.25 g of the aerial part sample in a microwave digestion container. In the next step, 0.1 mL of a solution of 50 $mg. L^{-1}$ of metals (Au + Lu) was added to each container. The containers were sealed and then transferred to a microwave system (Berghof Speed wave 4). The samples were digested at a minimum temperature of 190 °C for 10 min. In the next step, the containers were allowed to cool down to room temperature. Then the contents of each container were filtered and poured into a 50 mL HDPE centrifuge tube and then diluted with deionized water to a final volume of 20mL. To prepare the standards with a concentration range of each of the investigated elements, a preliminary analysis was performed and the required standards for each element were prepared with three repetitions. Digested samples were injected into HP 4500 ICP-MS device equipped

Table 1. Sequence of general primers (27F/1492R)

Gene	Numbers of N	Tm (°C)	Product length	Primer (5' 3')
27F	20	56	1464	5'-AGAGTTTGATCCTGGCTCAG-3'
1492R	19	52	1464	5'-GGTTACCTTGTTACGACTT-3'

with Asos-520 Autosampler (England) to measure elements (28).

3.5. Real Time PCR Reaction

RNA extraction from *A. absinthium* leaf tissue was done using the Trizol RNA extraction kit (Life Biolab, Germany) according to the manufacturer's instructions in a completely sterile and RNase-free condition. The quality and quantity of the extracted RNA were evaluated using gel electrophoresis and the detection of optical absorption ratio of the extracted RNA in the wave lengths of 260 to 280 nm, respectively. In order to be purified, the possible DNA in the extracted RNA was removed during the treatment steps with *DNase* I (Fermentas) according to the manufacturer's instructions. cDNA synthesis was performed by using BioFact™ RT cDNA synthesis kit (BioFact, Korea) according to the manufacturer's instructions. Real time PCR was performed in duplicate in a Step-One™ Real-Time thermocycler (Applied Biosystems, Foster City, CA, USA). The relative gene expression was calculated using $2^{-\Delta\Delta CT}$method. The design of PCR primers for the genes *CYP71AV1* (GenBank accession number: AB706290. 1) and ADS (GenBank accession number: LC106017. 1) was informed by the sequences obtained from the NCBI tool within the NCBI database. Sequencing results were checked by Chromas software and then evaluated using BLAST server in NCBI database for the identification of the species that attributed to each isolate. The present study investigates the relationship between absinthium and the conserved sequences of the 18S rRNA gene within a specific species. The primer sequences were developed utilizing Oligo software (version 7.6.0), and subsequently assessed for quality aspects such as the formation of loop structures, dimer formations, and other relevant characteristics using Oligo Analyzer software (version 1.0.2). Furthermore, the specific binding percentage of the designed primers to the target site was verified utilizing the BLAST tool. The synthesis of the compound was conducted by Arian Gene Gostar Company. The reaction mixture used contained cDNA with a final concentration of 1 ng.μL^{-1}, each of the forward (F) and reverse (R) primers with a final concentration of 0.25 pM (the sequences of which are listed in (**Table 1**), 10µL of a BioFact™ 2x Real time PCR master mix, 8µL of sterile distilled water, and Cybergreen dye; in a total volume of 20µL. Temperature program of the reactions for the target and reference genes (18S rRNA) amplification was included an initial annealing at 95 °C for 15 min, 45 cycles including annealing at 95 °C for 15 s, binding of primers at 60 °C for 25 s, extension at 72 °C for 35 s and final denaturation at 72 °C for 10 min. Also, the melting curve was drawn at the temperatures of 60 °C to 95 °C, with a 0.3 °C increase for each fluorescent reading (**Table 2**). The plant leaf samples were taken after 72 h to check gene expression and after 21 days to check other indicators including the secondary metabolite of Artemisinin (29, 30).

3.6. Statistical Analysis

This experiment was conducted in the form of a

Table 2. Specifications of primers used in Real Time PCR in Artemisia absinthium

Gene name	Primer sequence (5′ to 3′)	The length of products (bp)	Tm (°C)
18S rRNA	F:TAGAGCTAATACGTGCAACAAACC R:TAATTCTCCGTCACCCGTCACCA	216	59.3 63.0
Cytochrome P450 monooxygenase1 (CYP71AV1)	F:TGTCATGGGTGCAGAATACGAG R:ACCATTGGGGAGTTTCCAGTTG	139	60.4 60.8
Amorpha-4,11-diene synthase (ADS)	F:GAGGGACACATACCAACCACTGA R:AGAGACAGCCCATTCGACAGC	135	61.9 62.4

completely random statistical design at three salinity levels with three repetitions and the results were evaluated. The data analysis was carried out using SAS software, while the comparison of averages was performed using Duncan's test. Additionally, the graphs were generated utilizing Excel 2017 software.

4. Results

4.1. The Rhizosphere Bacteria

The isolated bacteria were identified as two *B. cereus* strains (E & B) based on the sequencing of the amplified 16S rRNA. The one of the sequences were deposited in

Table 3. Effect of combined treatment of rhizosphere bacteria (E & B) and salinity stress on plant growth parameters in Artemisia absinthium

Isolates	Salinity (mM)	Shoot length (cm)	Root length (cm)	Shoot (FW) (g)	Root (FW) (g)	Shoot (DW) (g)	Root (DW) (g)
Non application	0	$16.1±1.106^{b}$	$21.6±1.955^{b}$	$6.47±1.321^{b}$	$6.30±2.000^{bc}$	$0.82±0.297^{b}$	$0.39±0.202^{b}$
	75	$12.6±0.721^{c}$	$16.4±1.457^{cd}$	$3.92±1.447^{c}$	$3.79±0.884^{d}$	$0.61±0.310^{bc}$	$0.22±0.083^{bc}$
	150	$9.3±1.00167^{d}$	$14.2±0.100^{d}$	$1.75±1.030^{d}$	$2.95±0.202^{d}$	$0.2867±0.066^{c}$	$0.15±0.040^{c}$
application	0	$21.9±2.579^{a}$	$29.1±4.073^{a}$	$9.60±0.341^{a}$	$13.00±0.916^{a}$	$1.93±0.152^{a}$	$0.74±0.051^{a}$
	75	$17.1±1.824^{b}$	$18.8±1.501^{bc}$	$7.10±0.701^{b}$	$7.56±0.416^{b}$	$0.90±0.072^{b}$	$0.59±0.072^{a}$
	150	$12.6±2.165^{c}$	$16.1±0.984^{dc}$	$2.55±0.435^{dc}$	$4.60±1.053^{dc}$	$0.35±0.040^{c}$	$0.21±0.032^{bc}$

The combined effect of rhizosphere bacteria (E & B) and salinity stress (0,75,150 mM sodium chloride) on plant growth parameters in in *Artemisia absinthium*. The data is the average of 3 replications ± standard error (SE) and unlike letters indicated significant differences based on Duncan's test ($p \leq 0.05$).

Table 4. The effects of the combined treatment of rhizosphere bacteria (E & B) and salinity stress on the absorption of elements in *Artemisia absinthium*

Isolates	Salinity (mM)	Na^{+} (mg. g^{-1}DW)	K^{+} (mg.g^{-1}DW)	Ca^{2+} (mg.g^{-1}DW)	Mg^{2+} (mg.g^{-1}DW)	Fe^{2+} (mg.g^{-1}DW)
Non application	0	$3.94±0.167^{c}$	$6.60±0.371^{b}$	$3.23±0.170^{a}$	$1.65±0.220^{b}$	$0.12±0.028^{b}$
	75	$5.14±0.690^{b}$	$4.83±0.522^{c}$	$1.94±0.251^{bc}$	$1.08±0.175^{c}$	$0.05±0.006^{c}$
	150	$9.28±0.890^{a}$	$3.35±0.428^{d}$	$1.16±0.095^{d}$	$0.47±0.085^{d}$	$0.02±0.009^{c}$
application	0	$3.20±0.364^{c}$	$9.37±0.718^{a}$	$3.53±0.357^{a}$	$2.26±0.234^{a}$	$0.27±0.048^{a}$
	75	$3.70±0.630^{c}$	$6.64±0.338^{b}$	$2.26±0.192^{b}$	$1.47±0.205^{b}$	$0.15±0.026^{b}$
	150	$5.77±0.870^{b}$	$7.54±0.962^{b}$	$1.68±0.265^{c}$	$0.98±0.135^{c}$	$0.11±0.030^{b}$

The combined effect of rhizosphere bacteria (E & B (and salinity stress (0,75,150 mM sodium chloride) on the on the absorption of elements in *Artemisia absinthium*. The data is the average of 3 replications ± standard error (SE) and unlike letters indicate significant differences based on Duncan's test ($p \leq 0.05$).

GenBank with the accession numbers OQ410446 and OR473625.

4.2. Effect of Combined Treatment of Rhizosphere Bacteria E, B and Salinity Stress on Plant Growth Parameters in A. Absinthium

Bacteria cultured in nutrient agar plates did not show any inhibitory effect. The use of two bacteria together has a synergistic effect on the investigated indicators compared to when they are used individually. When the bacteria were used together, they did not show negative mutual effects on each other and on the plant.

The combined treatment of rhizosphere bacteria and salinity stress on root length was statistically significant and the combined treatment of rhizosphere bacteria and salinity stress on stem length was statistically significant. The biggest increase for stem length and root length is related to B. cereus (E & B (bacteria and 0 mM

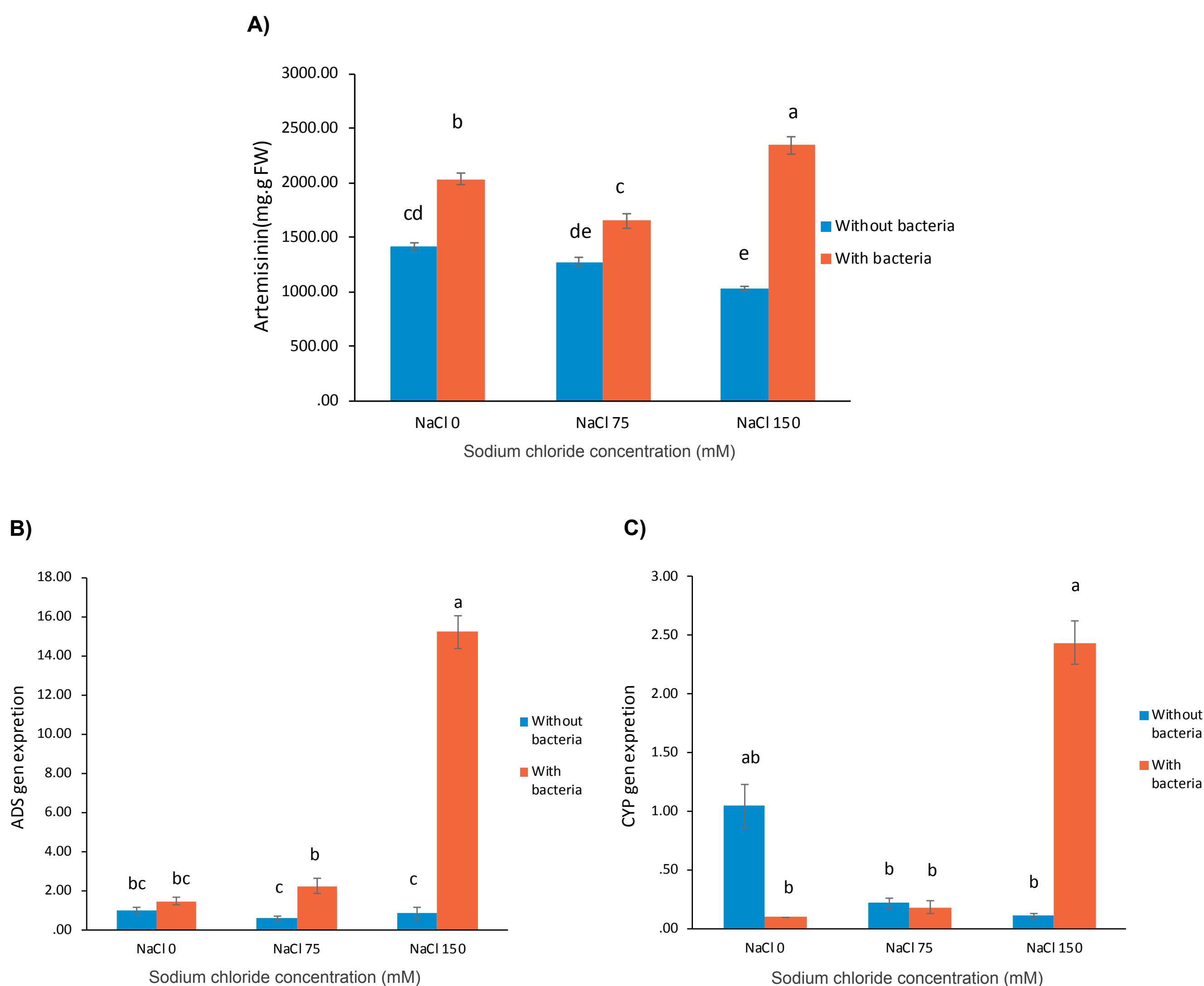

Figure 1. The effects of Combined treatment of rhizosphere bacteria (E & B) and salinity stress. A) *Artemisinin*. The data is the average of 3 replications ± standard error (SE) and unlike letters indicate significant differences based on Duncan's test ($p \leq 0.05$). **B)** *Amorpha-4,11-diene synthase* expression fold change; **C)** *cytochrome P450 monooxygenase1* expression fold change in *Art emisia absinthium.* The data is the average of 2 replications ± standard error (SE) and unlike letters indicated significant differences based on Duncan's test ($p\leq 0.05$).

Table 5. Correlation results between the amount of Artemisinin and the expression of two genes Amorpha-4,11-diene synthase and Amorpha-4,11-diene synthase under salinity stress and inoculation of *Artemisia absinthium* plant

	Correlation		
	Artemisinin	Amorpha-4,11-diene synthase	Cytochrome P4501 monooxygenase1
Artemisinin	1	0.540*	0.435
Amorpha-4,11-diene synthase	0.540*	1	0.726**
Cytochrome P4501 monooxygenase1	0.435	0.726**	1

salinity and this increase for stem is 0.36 and root 0.34 equal Control. The combined treatment of rhizosphere bacteria and salinity stress on stem and root wet weight was statistically significant. The highest increase for stem and root fresh weight is related to B. cereus (E &B (and 0 mM salinity, which is 0.48 for stem and 1.06 for root times equal Control. Treatment with rhizosphere bacteria and salinity stress was statistically significant on the dry weight of the root. The highest increase for the dry weight of the root is related to the combination of Bacillus cereus (E &B) and salinity of 0 mM and this increase is 0.9 times equal Control. Treatment with the combination of rhizosphere bacteria and salinity stress of 0mM on the dry weight of the stem was statistically significant and this increase for stem is 1.35 equal Control. The results are shown in **Table 3**.

4.3. The Effects of Combined Treatment of Rhizosphere Bacteria (E & B) and Salinity Stress on the Ion Homeostasis in A. Absinthium

The combined treatment of *Bacillus cereus* bacteria and salt stress on the absorption of potassium, calcium, magnesium, iron and sodium ions has been statistically significant. The combined use of *Bacillus cereus* bacteria (E & B) and salt stress increases potassium, calcium, magnesium and iron and decreased sodium ion. The highest increase is related to the combined treatment of rhizosphere bacteria and 0 mM salinity, which is equal control for potassium (0.42), calcium (0.093), magnesium (0.37) and iron (1.25) ions times equal Control and the least sodium ion is related to the combined treatment of rhizosphere bacteria and 0 mM salinity, this decrease is 0.162 times equal Control. The results are shown in **Table 4**.

4.4. The Effect of Combined Treatments of Rhizosphere Bacteria (E & B) and Salinity Stress on the Relative Expression of Genes and Artemisinin Production in A. Absinthium

The combined treatment of *A. absinthium* by the rhizosphere *B. cereus* strains E and B and salt stress caused an increased expression of *ADS* and *CYP71AV1* genes and an increased production of Artemisinin. The highest genes expression and Artemisinin production levels were significantly related to the combined treatment with *B. cereus* strains E and B and 150 mM sodium chloride compared to the control ($p \geq 0.05$). The results are shown in **(Fig. 1).**

Based on the data presented in **Table 5**, a statistically significant positive correlation is observed at the five percent level between the quantity of Artemisinin and the expression level of the ADS gene. Moreover, a statistically significant positive correlation was observed at a 99% confidence level between the expression levels of the two ADS genes and the *CYP71AV1* gene. There exists no discernible correlation between the levels of Artemisinin and the expression of the *CYP71AV1* gene. The combined treatment of bacteriorhizospheres (E & B) and 150 mM salinity stress caused a significant increase of 2.4-fold change in the level of *CYP71AV1* gene expression compared to the control without stress. The combined treatment of bacteriorhizospheres (E

and B) and 150 mM salinity stress caused a significant increase of 15.2-fold change in the expression level of *ADS* gene compared to the control without stress.

5. Discussion

Salinity stress caused a decrease in growth indices including stem and root length, stem and root weight, and stem and root dry weight, as shown in this study. The primary growth of plant roots is influenced by high salinity, resulting in restricted cell division and expansion. Additionally, when under salinity stress, the decline in auxin levels and the expression of auxin transporters cause a reduction in the activity of the root meristem, thus limiting the plant's initial growth (31). When considering the written composition, one suggests a shift towards a more academic mode of expression. In the present study, the concurrent application of rhizosphere bacteria (E) and (B) along with salinity stress was found to significantly enhance the elongation of stem and root, the biomass of shoot and root, and the dry mass of stem and root. One of the mechanisms utilized by growth-promoting bacteria, Plant Growth-Promoting Bacteria Plant growth-promoting bacteria (PGPB), to induce tolerance in plants towards diverse environmental stresses involves the regulation of phytohormone synthesis. Phytohormones play a crucial role in the plant's ability to withstand salinity stress by facilitating a protective response. This response results in enhanced cell proliferation within the root system, leading to an augmented root surface area capable of absorbing water and nutrients. Specifically, the increased production of root filaments contributes to the plant's improved capacity for water and nutrient absorption. Auxin, as one of the phytohormones, exerts a direct influence on the process of growth, thereby enhancing the uptake of nutrients and overall plant well-being during episodes of stressful environmental circumstances (32,33). According to recent studies, it has been asserted that minerals such as potassium (34), magnesium (35), and calcium (36) play a crucial role in facilitating the growth and development of plants. In the current study, notable observations were made regarding the amplification of element absorption under salinity stress through the application of combined treatment with rhizospheric bacteria. Hence, it can be posited that the aforementioned bacterial intervention enhances the assimilation of various components. Furthermore, it is plausible to assert that there exists the potential for further growth and expansion. This research investigation elucidates that in an environment exposed to salinity stress, the uptake rate of potassium, calcium, magnesium, and iron ions experiences a decrease, while the intake rate of sodium ions undergoes an increment. Under conditions of salinity stress, there is a significant increase in the concentration of Na^+ ions in plant tissue. This increase subsequently leads to alterations in the Na^+/K^+ ratio and inhibits the absorption of essential nutrients. The observed phenomenon can be ascribed to the competition arising from the comparable ionic radius of sodium and potassium within the soil. This competition consequently impairs the selective nature of the ion membrane. Furthermore, the augmented concentration of Na^+ ions in the soil area can disrupt the texture of the soil, thereby diminishing its porosity. Consequently, this reduction in soil porosity adversely affects both the soil's ability to facilitate adequate ventilation and its capacity for water conduction. Furthermore, it has been observed that regions characterized by the propensity for dehydration as a result of enhanced salt accumulation within the soil have the capacity to disrupt the process of water and nutrient absorption (37). This study observed a notable increase in the absorption of potassium, calcium, magnesium, and iron ions, as well as a decrease in the absorption of sodium ions, following the inoculation of bacteria (E) and (B) in the presence of salinity stress. In the present circumstance, microorganisms capable of dissolving potassium are deemed efficacious mechanisms for enhancing plant accessibility to this essential nutrient. The microorganisms exhibit the capability to transform elemental potassium into its mineral form, potassium ions (38, 39). The phenomenon of bacterial inoculation potentially enhances the uptake of potassium ions in a non-artificial manner, thereby leading to a reduction in the uptake of sodium ions. Consequently, this mechanism promotes a higher potassium to sodium ratio, ultimately facilitating ion equilibrium and the absorption of elements and water up to a certain extent. The status of the situation reverts to its original state. Bacteria have been found to employ the strategy of sequestering cations within the exopolysaccharide matrix. This phenomenon has been specifically observed in the *B. cereus* bacterium (40). Additionally, bacteria also influence the root structure by promoting rhizopod expansion and modulating the expression of transporters with ionic affinity. This, in

turn, facilitates the absorption of salt, particularly sodium chloride (NaCl), by the host plant (41, 42). The utilization of high-throughput plant growth-promoting bacteria (HT-PGPB) demonstrates its efficacy in mitigating the deleterious effects of salinity stress through enhanced nutrient uptake mechanisms within plant cellular structures. The process of Na^+ ion absorption by HT-PGPB occurs through alterations in the composition of the cell wall or cell membrane. The observed modifications entail an elevation in the expression levels of NHX, a proton/cation antiporter, and HKT, a high affinity potassium transporter. These altered expression levels are attributed to the plants' ability to selectively uptake potassium while concurrently transporting toxic sodium ions. This physiological response is accomplished through an upregulation of the salt-sensitive supersensitive gene (SOS) and an augmentation of the Na^+/H^+ electrogenic ion transporter (43). It is possible that the introduction of bacteria via the aforementioned techniques has led to a decrease in sodium absorption while concurrently resulting in an increase in potassium ions, water, and other relevant substances. In accordance with our research, a parallel observation has been made whereby the inoculation of wheat plants with *Bacillus subtilis* (GB03) resulted in a reduction in sodium ion levels and an enhancement in the potassium to sodium ratio, particularly in the presence of elevated salinity stress conditions (44). The application of *Bacillus* sp. in the simultaneous inoculation of *Solanum tuberosum* L., commonly known as the potato plant, was investigated in this study. The strains SR-2-1 and SR-2-1/1 exhibited a notable reduction in sodium ion levels and a concomitant increase in potassium ion concentrations, consequently leading to an elevation in the potassium to sodium ratio (45). The rice plant species, *Oryza sativa* L, was subjected to the process of inoculation using three distinct *Bacillus* strains, namely ALT11, ALT12, and ALT30, in the presence of salinity stress at concentrations of 70 and 140 mM NaCl. The experimental findings revealed a notable reduction in sodium ions, along with a concurrent elevation in potassium and magnesium ions. Additionally, the inoculation process resulted in an augmentation in both stem and root length of the rice plants (46). Inoculation of lettuce plants with *Pseudomonas* sp strains caused a significant increase in the concentration of potassium, the ratio of potassium to sodium, and the fresh and dry weight of the stem under different salinity levels (47). According to the researchers, the application of *B. subtilis* and *Pseudomonas fluorescens* to radish seeds resulted in a noteworthy augmentation of both the moist and dry biomass of roots and leaves. Furthermore, there was a substantial rise in the levels of nitrogen (N), phosphorous (P), potassium (K^+), calcium (Ca^{+2}), and magnesium (Mg^{+2}), while the amounts of sodium (Na^+) and chloride (Cl^+) experienced a decline, in comparison to the control group. The seed was exposed to salinity stress without any microbial inoculation (48). In a separate investigation, the introduction of *B. subtilis* RhStr_71, *Bacillus safensis* RhStr_223, and *B. cereus* RhStr_JH5 into pea plants (*Pisum sativum*) subjected to salt-induced stress resulted in a notable enhancement in stem and root length, as well as increases in the fresh and dry weights of both the root and stem (49). The results indicate a significant increase in the fresh and dry weight of both the root and stem of the inoculated plants, as compared to those subjected to salinity stress without bacterial inoculation (50). The inoculation of wheat plant (*Triticum aestivum* L) with *B. subtilis* bacteria resulted in a substantial enhancement in both root and stem development under salinity conditions (150- and 200-Mm sodium chloride), as compared to the control group without any inoculation (51). In the present study, the combined treatment of *B. cereus* B and E bacteria and sodium chloride salt stress increased the production of artemisinin and increased the expression of *ADS* and *CYP71AV1* genes compared to the control, which was statistically significant. Similar to our research, in research titled that the amount of Artemisinin in the inoculation of *A. annua* with a combination of four endophyte bacteria including *Bacillus subtilis, Bacillus licheniformis, Burkholderia* sp. and *Acinetobacter pittii,* was more than single cultures of these bacteria. This microbial combination was able to increase Artemisinin production by about 658% compared to the non-inoculated control. The use of endophyte microbial composition may be an effective alternative to chemical fertilizers to increase the performance of Artemisinin in an economical and environmentally friendly way by reducing its production cost (52). According to prior research, it has been theorized that *Bacillus* bacteria possess the capacity to engage in nitrogen fixation process. According to previous research findings, nitrogen is deemed vital for the productive synthesis and catalytic function of

terpene synthetase enzyme. *B. cereus* B and E bacteria are both able to dissolve phosphate and produce auxin hormone, but the amount of phosphate dissolution in *Bacillus* B bacteria and the amount of auxin hormone production in *Bacillus* E bacteria is high. Therefore, when both bacteria are inoculated together, it increases the rate of root growth and absorption of ions and water, increases phosphate dissolution (due to having the power of phosphate dissolution) to the optimum level and nitrogen fixation (considering that some PGPR bacteria stabilize are nitrogenizers and it has been stated that *Bacillus* bacteria have the ability to fix nitrogen (53). Moreover, the presence of the phosphate ion is imperative for the biosynthesis of terpenoid precursors and the generation of ATP and NADPH. Conversely, the phosphate ion also plays a crucial role in the process of terpenoid synthesis (54). It is possible that in this research, the use of these two bacterial strains has increased plant absorption of elements, including nitrogen and phosphorus, and enhanced enzyme activity and Artemisinin production. Inoculating stressed plants with microorganisms boosts secondary metabolite production. Microorganisms help plants fight stress by improving metabolic activities and producing secondary metabolites (55). *Bacillus* spp, known as growth promoting bacteria, have been found to exhibit promotion of growth in various contexts. The mediation of abiotic stress responses in plants has been observed, which frequently results in significant alterations in the levels, composition, and spatial distribution of primary and secondary metabolites (56).

6. Conclusion

The induction of salinity stress has been observed to result in a reduction in both the fresh and dry weight of the root and stem, as well as a decrease in the length of both the root and stem in *A. absinthium*. Additionally, there has been a decrease in the levels of potassium and calcium ions, magnesium, and iron, accompanied by an increase in sodium ion concentration. The utilization of the combined treatment of *B. cereus* E and B has demonstrated its efficacy in enhancing the ability of *A. absinthium* to endure salinity stress. This treatment has resulted in notable improvements in the fresh and dry weight of the plant's root and stem, as well as the length of these plant parts. Furthermore, the application of this treatment has led to significant increases in the concentrations of potassium, calcium, magnesium, and iron ions within *A. absinthium*. Additionally, it has caused a substantial augmentation in the production of Artemisinin, as well as the upregulation of two genes, namely *ADS* and *CYP71AV1*, which are part of the biosynthetic pathway for Artemisinin. Moreover, the combined treatment has been effective in reducing the levels of sodium ion in *A. absinthium*. Bacterial entities demonstrated the capacity to attenuate the impact of stress on the plant physiology. The findings of this study illustrate that bacteria possess the potential to function as an amplifying agent in augmenting Artemisinin levels in *A. absinthium*.

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Conflict of interest

The authors declare that there is no conflict of interest.

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