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Improved growth and salinity tolerance of the halophyte *Salicornia* sp. by co–inoculation with endophytic and rhizosphere bacteria

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ABSTRACT

The growth and yield of plants, including halophytes, are widely affected by salinity. It is known that the rhizosphere and endorhiza of the plants growing in saline environments harbor salinity–tolerant bacteria with plant growth promoting (PGP) potential. However, information about PGP endophytic and rhizosphere bacteria colonizing in the halophytes is still scarce. This study was designed to isolate and characterize rhizosphere and endophytic bacterial isolates from salt–accumulating halophyte *Salicornia* sp. grown under extreme salinity and to evaluate the effect of effective rhizosphere and endophytic bacterial strains (no bacteria, B0; rhizosphere strain *Staphylococcus* sp., R; endophytic strain *Staphylococcus* sp., E; and combination of these strains, R + E) on *Salicornia* sp.–plant growth promotion under salinity stress (0, 200, 400, and 600 mM NaCl) in greenhouse conditions. A total of 214 rhizosphere and endophytic isolates were obtained from rhizosphere soil and the surface–sterilized roots of *Salicornia* sp. These isolates exhibited tolerance to NaCl and drought and had different PGP traits. The results of this study also showed that the growth of the halophyte *Salicornia* sp. was also affected by salinity. At level of 200 mM NaCl, salinity had a positive effect on plant growth, so that the highest growth indices of the plant were observed at this salinity level. However, at salinity levels higher than 200 mM NaCl (400 and 600 mM NaCl), the growth of the plant (5.8–42.9% decrease in growth indices) decreased compared to that of this plant at the concentration of 200 mM NaCl. Bacterial strains (R, E, and R + E) had a positive role in alleviating the negative effects of salinity on the plant growth (13.9–47.0% increase in growth indices) compared to control (B0) at all salinity levels. In the presence of the bacterial strains (R, E, and R + E), the highest plant growth (33.2–65.2% increase in growth indices) was obtained at a concentration of 200 mM NaCl. Among bacterial treatments, the combination of these strains (R + E) had the highest effect on the plant growth under salinity stress. In general, the results of this study show that salinity–tolerant rhizosphere and endophytic bacteria associated with the halophyte *Salicornia* sp. have an important role in enhancing growth and salt tolerance in the halophyte *Salicornia* sp. and can be used as bio–fertilizer for further improvement of the growth of this plant in saline environments.

1. Introduction

Salinity is one of the abiotic stresses that greatly affect the yield of salinity–sensitive plants, microbial communities and even halophyte plants through affecting their physiological, biological and metabolic processes (Sobhanian et al., 2011; Szymańska et al., 2018). Due to the decrease in the level of fertile land, saline soil–based agriculture has been developing rapidly in recent years (Zhu et al., 2011). A restriction to this agricultural approach is low salt tolerance of agricultural crops and trees (Glenn et al., 1991). In such saline soils, the plants that are salt–resistant can produce significant yields. Halophytes are highly salt–resistant plants that can grow in areas with high levels of salinity (from 200 mM NaCl) (Flowers and Colmer, 2008), where no cultivation occurs. These plants, with a wide range of physiological, morphological and biochemical mechanisms, can adapt and grow in high–salinity soils (Flowers and Colmer, 2008; Flowers et al., 2010). Halophytic plants evolved various strategies to live in a saline environment. These strategies include raising the osmotic pressure in the cytoplasm, the production of compatible solutes, the exclusion of Na^+ from cells, and the accumulation of Na^+ in the vacuole (Flowers and Colmer, 2008).

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Despite these mechanisms, it is known that the initial establishment of halophyte seed is delayed under conditions of high salt stress (Song et al., 2008). Studies have shown that salt–tolerant rhizospheric and endophytic bacteria associated with roots of halophyte plants contribute to salinity tolerance of these plants and cause them to grow better under salt stress conditions (Etesami and Beattie, 2018). Furthermore, it is reported that stress tolerant–plant growth–promoting bacteria (PGPB) are able to promote the systematic tolerance of plants by inducing the physical and chemical changes of their hosts that result in tolerance improvement to abiotic stress (Kohler et al., 2009). The phenomenon of habitat–adapted symbiosis is an example of an adaptation of plants to adverse environments through the symbiosis with stress tolerant–microorganisms (Rodriguez et al., 2008).

Salt tolerant–bacteria have the ability to grow in the concentrations of 1–33% NaCl, and using different mechanisms, they can overcome the stress caused by high salt concentrations (Larsen, 1986). Based on area of colonization, bacteria can be grouped into associative bacteria that include rhizosphere (in vicinity of root), rhizoplane (on surface of root), and endophytic bacteria (isolated from the interior of tissues) (Sturz et al., 2000). Endophytes including bacteria living asymptomatically within plant tissues (i.e., stem, root, seed, leaf, etc.) have been found in almost all plant studies to date (Hallmann et al., 1997). In comparison to rhizosphere, endophytic bacteria showed better adaptations against biotic and abiotic stresses, which result in enhanced plant growth (Pillay and Nowak, 1997). Many endophytic bacteria constitute the common rhizospheric bacteria that produce various plant growth promoting (PGP) secondary metabolites, (Lodewyckx et al., 2002). Similar to the mechanisms of rhizosphere bacteria, endophytic bacteria can increase their host plant growth (Lugtenberg and Kamilova, 2009).

The role of salt tolerant–rhizosphere and endophytic bacteria in the plant promotion by various mechanisms has been well reviewed (Etesami and Beattie, 2018; Etesami and Maheshwari, 2018).

In recent years, PGP–rhizosphere and endophytic bacteria associated symbiotically with halophytes have been studied (Etesami and Beattie, 2018; Jha et al., 2012; Zhao et al., 2016), but data are still limited. Improved knowledge on the microbiota associated with halophytic plants (native habitat–adapted symbiotic bacteria) increases understood salt–tolerance of halophytic plants (Yuan et al., 2016) and may help elucidate their functions and potential role in improving the performance of halophytes and contribute to their host salt –tolerance ability (Zhao et al., 2016).

Salicornia, belonging to family Amaranthaceae, is one of the most important halophyte (salt –tolerant) plants with the highest salinity tolerance (one of the most salt–accumulating halophytes known) (Davy et al., 2001). This plant is of commercial value and ecological importance. Research has shown that *Salicornia* has a high ability to produce oil–rich seeds. The seed of this plant contains 28% oil. In addition, *Salicornia* is also used in medicine for its antimicrobial properties. Re–vegetation of saline soils with halophytes is regarded as a proactive phytoremediation method to improve soils (El Shaer, 2010). The halophyte *Salicornia* has been cultivated for soil desalination because it hyperaccumulates salt in the saline soil (Hasanuzzaman et al., 2014). Hence, improved adequate rate of seed germination and a sufficient quantity of biomass of the halophyte *Salicornia* in high salt–-concentrations by using salinity tolerant–bacteria associated with this plant was important. These bacteria could be applied as bio–inoculants to facilitate salt soil phytoremediation and be beneficial for mitigating the salt stress to the plants growing in such salt–affected habitat. There have been a few reports of beneficial bacteria being associated with *Salicornia* species i.e., *S. europaea* (Zhao et al., 2016), *S. brachiata* (Jha et al., 2012), and *S. strobilacea* (Mapelli et al., 2013; Marasco et al., 2016); however, to date, there have been no published works about the combined use of salinity tolerant–rhizosphere and endophytic bacteria associated with this halophyte to improved growth under saline conditions. Since the survival of single species of beneficial bacteria is often hampered by various reasons, it was hypothesized that it is a combination of various bacteria that together would exert beneficial effects (Qin et al., 2016).

Therefore, this study addressed two fundamental questions: (i) how do the traits of bacterial isolates (e.g., resistance to drought and salinity and PGP traits) differ between the rhizosphere and endorhiza of the halophyte *Salicornia* sp. native to Iran?, and (ii) if rhizosphere and endophytic bacteria associated with the halophyte *Salicornia* sp. have a role in improved salt tolerance of this plant? If so, which one has the highest effect? (endophytic strain or rhizosphere strain or combination of both). In other words, the goal was to find out if the salt –tolerance comes from genetic traits of the plant or not. In order to address these questions, all culturable rhizophere and endophytic bacterial isolates isolated from the halophyte *Salicornia* sp. were screened for their resistance to drought and salinity and PGP traits. In addition, the single and combination effect of an effective rhizophere strain and an effective endophytic strain on improvement of growth and salt tolerance in the halophyte *Salicornia* sp. under salinity stress was studied.

2. Materials and methods

2.1. Sampling and isolation of rhizoshere and endophytic bacteria

Samples were taken from three regions of Qom (51° 10′ East longitude, 35° 19′ North latitude and 958 m altitude), Arak (49° 48′ East longitude, 34° 10′ North latitude and 1672 m altitude) and Eshtehard (50° 29′ East longitude, 35° 44′ North latitude and 1158 m altitude), Iran. At the flowering stage of the halophyte *Salicornia* sp., 12 healthy plant samples (from each site, four plants were chosen at random) and 12 soil samples from each region were selected and transferred to the laboratory for laboratory analysis at 4 °C. Electrical conductivity (EC) and pH of the soil of the region of Qom, Arak, and Eshtehard were 61 dS m^{-1} and 7.2, 75 dS m^{-1} and 6.9, and 45 dS m^{-1} and 7.9, receptively. Isolation of rhizoshere and endophytic bacterial isolates from the halophyte *Salicornia* sp. was carried out according to a previous report (Szymańska et al., 2016). Briefly, to isolate the endophytic bacterial isolates, healthy roots from the *Salicornia* sp. plant were carefully rinsed free of soil under running water, put in a fine–mesh plastic holder and surface–sterilized by immersion in 15% hydrogen peroxide (H_2O_2) for 5 min with shaking and finally rinsed five times with 100 mL of 2% NaCl solution. To confirm that the surface–sterilization process was successful, the surface–sterilized roots were rolled on nutrient agar (NA) (5.0 g L^{-1} of peptone, 1.5 g L^{-1} of yeast extract; 1.5 g L^{-1} of beef extract, 5.0 g L^{-1} of NaCl, 20 g L^{-1} of agar, pH 7.2) culture medium and the aliquots of the sterile distilled water from the final rinse solutions were plated onto NA plates as controls to detect possible contaminants. Roots without growth on the control–plates were considered as effectively surface–sterilized and the NA plates were used for the isolation of root endophytic bacteria. The surface–sterilized and fragmented roots (10 g) were crushed in a sterile mortar under sterile conditions. After preparing serial dilutions (10^{-1} to 10^{-5}) in 2% NaCl solution from the samples, 0.1 mL of each dilution was spread on plates containing NA culture medium supplemented with 2% NaCl. To isolate the rhizospheric bacterial isolates, 10 g of roots containing rhizospheric soil were transferred to the sterilized Erlenmeyer flask containing 90 mL of sterile 2% NaCl solution and placed on a shaker (at 120 rpm) for 30 min. The serial dilutions for rhizosphere soil were similar to root serial dilutions. The 0.1 mL of each dilution was spread on plates containing NA culture medium supplemented with 2% NaCl. All plates inverted were placed for 7 days at 28 ± 2 °C in an incubator and the number of colonies appearing on the plates was counted. The number of endophytic and rhizospheric bacterial isolates was reported as colony–forming units (CFU) g^{-1} fresh root weight and soil weight, respectively. The purity of the isolates was checked by repeated streaking of single colonies on the same media and by microscopic examination (i.e., color, shape, motility, growth rate, colony morphology, and Gram–staining). Pure cultures were preserved at 4 °C for

temporary storage or in nutrient broth (NB) containing 20% v/v glycerol at −80 °C for long–term storage.

2.2. Preparation of bacterial inoculum

The isolates were grown in NB medium, under agitation at 1200 rpm for 2 days at 28 ± 2 °C (log phase). Each suspension was centrifuged (2500*g*, 15 min, room temperature), re–suspended in sterile distilled water and adjusted to a final concentration of 5×10^8 CFU mL^{-1} for use as inoculum for all of the following assays.

2.3. Salt tolerance of bacterial isolates

NaCl tolerance test was examined at different NaCl concentrations (0, 5, 10, 15, 20, and 25% w/v) on NA medium. The isolates (logarithmic phase was attained 5×10^8 CFU mL^{-1}) were spot–inoculated on the respective plates. Each treatment was repeated three times and the plates were incubated in an incubator at 28 ± 2 °C for 48 h. Then changes in diameter, status, and appearance of colonies were investigated, compared (with control), and recorded. In this study, the isolates that could grow on medium supplemented with ≥15% NaCl were considered salt–tolerant strains and were further characterized *in–vitro* for drought tolerance and PGP traits.

2.4. Drought tolerance of salt–tolerant isolates

To evaluate the tolerance of isolates to different levels of drought, their growth potential in NB medium containing concentrations of 0, 202.2, 295.7, and 367.7 g polyethylene glycol (PEG)–6000 L^{-1} was tested. Osmotic potential (ψs) of the aqueous solutions of PEG–6000 mentioned above was 0, −5, −10, and − 15 bar, respectively (Michel and Kaufmann, 1973). The growth rate of isolates was determined by measuring optical density (OD) at 630 nm with a Microplate Reader (Bio–Tek Elx800, USA) and the percentage of growth of each isolate at different levels of PEG–6000 was compared with the amount of the growth of the same isolates in the NB medium without PEG and was then calculated. The experiments were done in triplicates. Three replicates from a un–inoculated NB medium containing different amounts of PEG–6000 were also provided as control to determine the OD of this medium.

2.5. Assays for PGP traits

2.5.1. Indole–3–acetic acid (IAA) production assay

IAA production was analyzed according to Patten and Glick (1996) with modifications. The isolates were previously grown in 20 mL Tryptic Soy Broth (TSB) medium supplemented with 100 μg mL^{-1} L–tryptophan for 24 h, at 28 ± 2 °C under agitation at 120 rpm. Bacterial suspensions were centrifuged for 15 min at 2500*g* at room temperature. The supernatant was combined with Salkowski's reagent (1:2; v/v) containing 150 mL H_2SO_4, 250 mL H_2O, and 75 mL 0.5 M $FeCl_3 \times 6H_2O$, and incubated for 30 min at room temperature in the dark. Non–inoculated broth served as control. The formation of pink color in test tubes indicated IAA production. Absorbances were read by a spectrophotometer (model of Shimadzu–UV3100) at 530 nm from three replicates per isolate, and IAA levels were estimated in relation to the standard calibration curve (with a range of 1–40 μg mL^{-1}) of the hormone.

2.5.2. 1–aminocyclopropane–1–carboxylate (ACC)–deaminase production assay

ACC–deaminase (E.C. 4.1.99.4) production was analyzed according to a previous method (Penrose and Glick, 2003) with modifications. The isolates were previously cultured in 20 mL NB medium for 24 h at 28 ± 2 °C, centrifuged (20 min; 2500*g*) and washed twice with Dworkin and Foster (DF)–salts minimal medium (Dworkin and Foster, 1958) without glucose and nitrogen (N) salts. The pellet was re–suspended in 10 mL of liquid DF–salts medium without N and agitated for 48 h at 100 rpm and at 28 ± 2 °C. The ACC–deaminase assay comprised three treatments with different N source media: solid DF–salts medium supplemented with 3 mmol L^{-1} ACC as the only N source, solid DF–salts medium without any sources of N (DF–N) as negative control, and DF–salts medium containing N (DF complete; DF + N) as positive control. Isolates aliquots (7 μL; 5×10^8 CFU mL^{-1}) were spot–inoculated in triplicate per treatment and sterile distilled water was used as the control. Plates were incubated at 28 ± 2 °C for 72 h. The qualitative assessment was based on the growth of each isolate, and it was considered positive for ACC–deaminase production for the isolates that grew in the medium with ACC and showed no growth on DF–N.

2.5.3. Phosphate solubilization assay

The bacterial isolates were screened for their inorganic phosphate–solubilizing ability on medium (glucose, 10 g; yeast extract, 0.5 g; $CaCl_2$, 0.1; $MgSO_4.7H_2O$, 0.25; $Ca_3(PO_4)_2$, 2.5 g; agar, 15 g; pH, 7.2; and distilled water, 1000 mL) proposed by Sperber (1958) with taking the suggestion of Bashan et al. (2013) into account. Each isolate was assayed by spotting 7 μL of cultures (logarithmic phase was attained 5×10^8 CFU mL^{-1}) on the plated media. After seven days of incubation (DOI) at 28 ± 2 °C, the plates were examined for the presence of colonies developing clear haloes and the halo and colony diameters were measured. The ratio of the total diameter (colony + halo zone) to the colony diameter was considered as a solubilization index (SI) for evaluating the insoluble phosphate–solubilizing isolates.

2.6. Germination rate test

Before doing the greenhouse experiment, the effect of the effective isolates (resistant to 15% NaCl and drought and having multiple PGP traits) on germination rate of seeds of the *Salicornia* sp. was studied. *Salicornia* sp. mature seeds, obtained from the Seed and Plant Improvement Institute (SPII), were surface–disinfected by rinsing the seeds with 70% ethanol for 20 s followed sodium hypochlorite (5% w/v) for 10 min and repeated washing with sterile deionized distilled water (Bashan and Holguin, 1997). In order to inoculate the seeds with the bacterial isolates, seeds were immersed into the broth culture of each bacterial isolates for 4 h. Seeds immersed in sterilized broth served as the control. Three replicates for each treatment containing 20 seeds per replicate were used. Seed germination was carried out in 9.5 cm–Petri dishes including 1% water agar. The inoculated and non–inoculated seeds placed on Petri dishes were incubated at 22 °C. Seeds were considered germinated when the root was at least 2 mm long. The seed germination rate was counted after seven days and compared with the control (without bacterial inoculum).

2.7. Molecular identification of effective isolates

Effective isolates selected were subjected to taxonomic identification by using 16S rRNA gene sequencing. Pure cultures in 10 mL liquid NB medium were prepared and incubated at 28 ± 2 °C for 2 days (exponential phase). The genomic DNA of the isolates was extracted using isolation kit (Promega, Madison, WI, USA). Quality and integrity of the DNA were determined by electrophoresis in 0.8% agarose gel and visualized by UV light. The amplification of 16S rRNA gene was done using universal primers 27F and 1492R following the protocol described by Edwards et al. (1989). The PCR products were sequenced at Macrogen Inc., Republic of Korea, and the obtained sequences were compared to those from the GenBank using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The nucleotide sequences identified in this study were sent to GenBank databases and recorded with various accession numbers.

2.8. Experimental set–up for pot experiment

To determine the effect of two effective bacterial isolates, one endophytic isolate and one rhizosphere isolate, on improved resistance of the halophyte *Salicornia* sp. to salinity, a pot experiment in a completely randomized design (CRD) with factorial arrangement (4 × 4) with three replications in a research growth chamber, located at Soil Science and Engineering Department of University of Tehran, Iran, was designed and implemented. Excremental treatments included: salinity factor at four levels, 0, 200, 400, and 600 mM NaCl, and bacterial factor at four levels, no bacteria (B0), rhizospheric isolate (R), endophytic isolate (E), and rhizospheric isolate plus endophytic isolate (E + R).

Plastic pots without drainage (19 × 19 cm) were used for this assay. The pots were disinfected with 75% sodium hypochlorite solution and rinsed several times in sterile distilled water. These pots were filled with 3.5 kg dry soil (non–sterile soil) passed through a 4–mm sieve. The soil used in this assay had a texture of sandy loam with 16.32% clay, 23% silt, and 60.68% sand; pH, 7.8; EC, 1.10 dS m^{-1}; organic carbon, 3.0 g kg^{-1}; total nitrogen, 0.46 g kg^{-1}; NH_4OAc–K, 225 mg kg^{-1}; Olsen-P, 8.8 mg kg^{-1}; calcium, 18.4 mg kg^{-1}; magnesium, 4.6 mg kg^{-1}; sodium, 6.0 mg kg^{-1}; iron, 4.0 mg kg^{-1}; and saturation percentage (SP) of soil, 26. According to a previous method (Okalebo et al., 2002), these traits were measured.

For their use in the experiments, the seeds were surface–disinfested as mentioned above and treated with the isolates. For the bacterization, the seeds were immersed in 10 mL of bacterial suspension per treatment (R, E, and E + R) for 4 h and partially dried for 1 h in un–covered petri dishes in a laminar flow hood. For the control, seeds were kept in sterile distilled water and dried at the same conditions. After testing the compatibility of the both bacterial isolates with each other according a previous report (Etesami et al., 2014), consortium of the cultures was prepared by mixing equal volume of the grown bacterial cultures (R and E). Before sowing the inoculated and un–inoculated seeds in the potted soil, they were first germinated in trays containing sterilized coco–peat for 20 days. Ten seedlings were sown into the potted soil and transferred aseptically to the research growth chamber (with 65% relative humidity and 100 µmol photons m^{-2} s of light). The length of the light and dark period in the first 50 days was 14 and 10 h, respectively, and the remaining time of cultivation was 16 and 8 h, respectively. The day and night temperature were 24 and 18 °C, respectively. Two weeks after sowing in the pot, the number of plants was reduced to three plants per pot and salinity treatments were applied. To avoid salt shock, NaCl solutions were applied to pots in increments of 100 mM NaCl every 48 h, until final salt concentrations of 200 mM NaCl (two times irrigation during two days), 400 mM NaCl (four times irrigation during four days), and 600 mM NaCl (six times irrigation during six days) were reached. Irrigation of plants was carried out with distilled water until saturation conditions were achieved (based on saturation percentage of soil). Also due to soil test, the deficiency of the nutrients was corrected through Hoagland solution and with irrigation water (distilled water).

2.8.1. Measurements

Plant biomass was recorded after harvesting at 100 days. The plant samples (shoot and root) were dried in an 80 oven for 72 h and the dry weight was recorded. In addition, root length, plant height, and the ratio of root to shoot dry weight (by dividing root values by shoot values) were also measured. Oven–dried plant samples (vegetative parts) were ground and 0.5 g of the samples digested with a mixture HNO_3:$HClO_4$:H_2SO_4 (8:1:1, v/v) at 150 °C for 45 min; mixed with 2 M HCl and filtered with Whaman No. 41. After the filtration, distilled water was added to make the volume 100 mL. Na^+ and K^+ contents were determined in the digested samples by flame photometer (BWB XP, BWB Technologies Ltd. UK) (Ryan et al., 2007). The K^+/Na^+ ratio was also calculated by dividing K^+ concentration by Na^+ concentration. According to a previous method (Weatherley, 1950), leaf relative water content (RWC) was determined in the first fully expanded leaves and total Chlorophyll (Chl) was determined in the leaves by extracting with dimethyl sulphoxide according to the non–maceration method of Hiscox and Israelstam (1979) using the Arnon's equation (Arnon, 1949). The proline content was estimated according a previous method (Bates et al., 1973). The 0.3 g fresh leaf samples were homogenized in 10 mL of 3% sulfosalicylic acid with a mortar. The 2 mL of homogenate was filtered and equal volume of 2 mL glacial acetic acid and 2 mL ninhydrin was added to the filtrate. The mixture was kept in 100 °C water bath for 45 min. Later, proline was separated with 4 mL toluene and absorbance was measured at 520 nm with toluene as blank by spectrophotometer (Cary 300 UV–Vis). The proline content of the samples was calculated in µmoL g^{-1} fresh weight (FW). To determine the activity of superoxide dismutase (SOD), as described by Giannopolitis and Ries (1977), the samples of fresh leaves (0.1 g) were placed into a 2 mL tube and frozen in liquid nitrogen. The samples were homogenized with a mortar in 100 mM phosphate buffer (pH 6.8) containing 0.1 mM EDTA and 1% PVP (polyvinylpyrrolidone). After that the homogenate was centrifuged at 12000 × *g* for 20 min at 4 °C, the supernatant was utilized for enzymatic assay of SOD (EC 1.15.1.1). The SOD activity was measured at 560 nm by spectrophotometer (Cary 300 UV–Vis) via monitoring the decrement in the absorbance due to the photochemical reduction of nitro blue tetrazolium (NBT). One unit of SOD was defined as the required amount for the enzyme reaction depending on 50% inhibition of NBT reduction based on the measurements at 560 nm. The SOD activity was calculated in unit mg^{-1} FW. Salt tolerance index (STI) was also calculated by dividing the shoot dry weight of plants inoculated/non–inoculated with bacterial strains under salinity stress by the shoot dry weight of plants non–inoculated with bacterial strain under non–saline conditions.

2.9. Statistical analysis

Data were analyzed by two–way analysis of variance (ANOVA) for the main effects (salinity levels and bacterial strains) and their interactions using SAS computer programs (SAS Institute, Cary, NC, USA). Significant differences among means were tested with Tukey's test at $P < 0.05$. The data represented in the tables and figures are expressed as means of three biological replicates ± standard deviation (SD).

3. Results

3.1. Endophytic and rhizosphere isolates

A total of 214 bacterial isolates (151 rhizosphere isolates and 63 endophytic isolates) with different phenotypes were isolated from the halophyte *Salicornia* sp. Out of 151 rhizosphere isolates, 64 isolates (1.5×10^7 CFU g^{-1}), 34 isolates (9.9×10^6 CFU g^{-1}), and 53 isolates (1.2×10^7 CFU g^{-1}) were isolated from *Salicornia* sp. plants grown in region of Qom, Arak, and Eshtehard, respectively. In addition, out of 63 endophytic isolates, 32 isolates (1.3×10^5 CFU g^{-1}), 20 isolates (7.2×10^3 CFU g^{-1}), and 11 isolates (6.3×10^3 CFU g^{-1}) were isolated from *Salicornia* sp. plants grown in region of Qom, Arak, and Eshtehard, respectively.

3.2. Tolerance of isolates to salinity

According to the results of the tolerance assay of isolates to salinity stress, out of 214 isolates, 98.1, 83.6, 52.3, 23.4, 7.0, and 3.3% were able to grown at a concentration of 0, 5, 10, 15, 20, and 25% NaCl, respectively (Fig. 1). In this assay, 1.9% of bacterial isolates were not able grow at 0% NaCl level. This shows that these bacteria were probably obligate halophytes (need for at least 2% NaCl). Of the 214 isolates, rhizospheric isolates had higher salinity tolerance than endophytes. On the other hand, comparison of the frequency of endophytic and rhizospheric bacterial isolates in the three sampled regions indicated that the Arak regional isolates, which had the highest

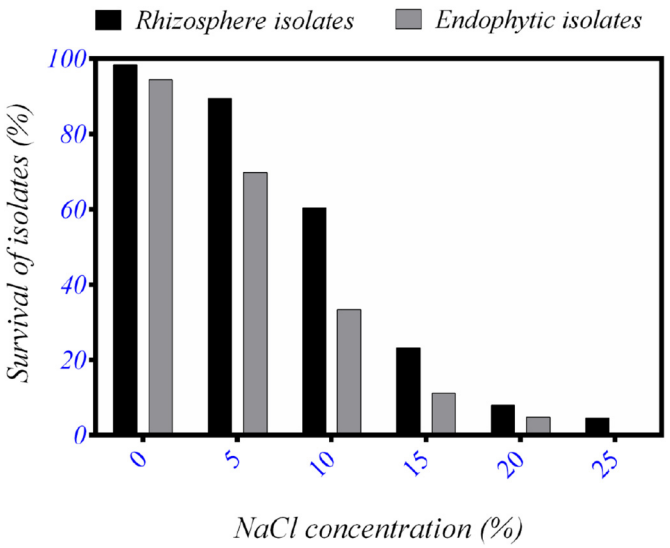

Fig. 1. Bar chart to show salinity tolerance of 214 bacterial isolates (151 rhizosphere isolates and 63 endophytic isolates) isolated from rhizosphere soil and surface–sterilized roots of salt–accumulating halophyte *Salicornia* sp.

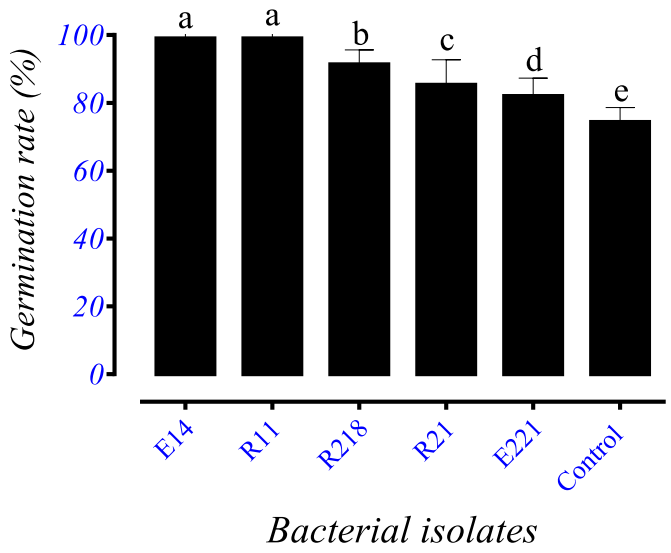

Fig. 2. The effect of five effective rhizosphere (R11, R218, and R21) and endophytic (E14 and E221) isolates on germination rate of seeds of the halophyte *Salicornia* sp. Data represent the mean ± SD (n = 3) and different letters indicate a significant difference ($P < 0.05$) using Tukey's test.

EC (75 dS m^{-1}), had greater abundance at different levels of salt concentration, and in contrast, the lowest frequency was related to endophytic bacterial isolates in Qom region (data not shown). Among 15% NaCl resistant isolates, only 15 isolates (ten rhizosphere isolates R21, R22, R37, R211, R11, R216, R218, R351, R350, and R335 and five endophytic isolates E23, E36, E122, E221, and E14) were selected for test of drought resistance. These 15 isolates had a faster growth rate than other isolates.

3.3. Tolerance of isolates to drought

Of the 15 selected isolates, 80, 46.7, and 20% grew at osmotic pressure of −5, −10, and −15 bar, respectively. In this assessment, the resistance of rhizospheric bacteria to drought was also higher than that of endophytic bacteria to drought. Of the five endophyte isolates (E23, E36, E122, E221, and E14), every five isolates grew at −5 bar osmotic pressure, but out of ten rhizospores isolates, only seven isolates grew at this osmotic pressure. At −10 bar osmotic pressure, out of five endophytic isolates and 10 rhizosphere isolates, three and four isolates grew, respectively. At −15 bar osmotic pressure, out of five endophytic isolates and 10 rhizosphere isolates, one and two isolates were able to grow, respectively.

3.4. Assay of PGP traits

For PGP traits characterization, the results showed that all of the 15 selected bacterial isolates had the ability to produce IAA, although production differed among the isolates. The amount of IAA production in the five endophytic isolates was between 1.3 ± 0.12 to 2.9 ± 0.30 μg mL^{-1}, while the production amount of this hormone in the rhizosphere isolates was between 1.4 ± 0.3 to 3.9 ± 0.4 μg mL^{-1}. Out of five endophyte isolates, three isolates had the ability to dissolve inorganic phosphate (SI = 1.4–2.6), while out of ten rhizosphere isolates, eight isolates had the ability to dissolve organic phosphate (SI = 1.4–3.5). Only two endophytic isolates and three rhizosphere isolates showed growth on the medium supplemented with ACC as the only source of N, indicating activity of the enzyme ACC–deaminase.

3.5. Germination rate test

Five ACC deaminase–producing endophytic and rhizosphere isolates (E14, E221, R11, R218, and R21), which also had the highest amount of IAA production and SI, were selected for germination test. These bacterial isolates significantly ($P < 0.05$) affected the final percent germination of seeds, and the highest value (33.2% compared to control) was recorded in the seeds inoculated with isolates E14 and R11 (Fig. 2). According to the results, endophytic isolate E14 and rhizospheric isolate R11 were selected for identification and pot experiment.

3.6. Identification of E14 and R11 isolates

The partial 16S rRNA gene sequences of both E14 and R11 showed 99% similarity with *Staphylococcus* sp. sequences available in public domain databases. Endophytic isolate E14 and rhizosphere isolate R11 were assigned as *Staphylococcus* sp. E14 and *Staphylococcus* sp. R11, respectively. The nucleotide sequences identified in this study were sent to the GeneBank database and recorded with accession numbers MG865740 and MG865739 for strains E14 and R11, respectively. Both strains showed substantial potential for inorganic phosphate solubilization and were positive in terms of ACC–deaminase activity. Endophytic isolate E14 and rhizosphere isolate R11 produced 2.9 and 3.9 μg mL^{-1} IAA, respectively. Both strains were able to grow at 20% NaCl and − 15 bar osmotic pressure.

3.7. The effect of strains E14 and R11on plant morphological parameters

With increasing the concentration of NaCl under conditions of without bacterial inoculation (B0), the plant height also increased and the highest value (25.5% relative to the concentration of 0 mM NaCl) was obtained at a concentration of 200 mM NaCl (Table 1). Inoculation of bacterial isolates increased plant height at all salinity levels. At the concentrations of 0 and 200 mM NaCl, the co–inoculation of rhizosphere and endophytic isolates increased plant height by 71.77 and 88.9% compared to B0, respectively, while the co–inoculation of these isolates increased plant height by 72.2 and 85.7% compared to control (B0) at the concentrations of 400 and 600 mM NaCl.

Application of salinity stress in conditions of without inoculation of bacterial isolates (B0) reduced root length at all salinity levels (Table 1). With increasing NaCl concentration, the difference between control treatments (B0) and bacterial treatments increased and peaked at 600 mM NaCl. Plants inoculated with strains R11 and E14, and consortium of both strains in both normal soil and salt–affected soils caused higher root length of *Salicornia* sp. (26.8–86.5%) as compared to

Table 1
Mutual effects of treatments (levels of salinity and bacterial strains) on morphological parameters of the halophyte *Salicornia* sp. The plants were cultivated for 100 days in potted soil under greenhouse conditions.

Treatments		Plant height (cm)	Root length (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Root dry weight/shoot dry weight ratio
0 mM NaCl	B0	7.2 ± 0.8 h	9.3 ± 1.5 cdef	0.76 ± 0.01 e	0.12 ± 0.00 fg	0.14 ± 0.00 f
	R	11.3 ± 1.3 cdefg	12.0 ± 1.7 abc	0.90 ± 0.10 de	0.14 ± 0.00 e	0.17 ± 0.01 cd
	E	11.2 ± 1.3 defg	10.0 ± 1.0 bcdef	0.97 ± 0.07 d	0.13 ± 0.01 ef	0.17 ± 0.02 cd
	R + E	12.3 ± 2.0 cde	10.5 ± 2.3 bcd	0.95 ± 0.14 d	0.16 ± 0.01 cd	0. 18 ± 0.02 b
200 mM NaCl	B0	9.0 ± 1.0 fgh	9.3 ± 1.5 cdef	0.86 ± 0.11 de	0.13 ± 0.01 ef	0.15 ± 0.00 e
	R	10.7 ± 1.3 defg	10.3 ± 0.6 bcde	1.15 ± 0.04 c	0.15 ± 0.00 d	0.18 ± 0.02 b
	E	16.2 ± 1.7 ab	11.8 ± 1.0 abcd	1.53 ± 0.12 b	0.18 ± 0.01 ab	0.20 ± 0.01 a
	R + E	17.0 ± 2.0 a	11.8 ± 1.9 abcd	2.18 ± 0.17 a	0.19 ± 0.01 a	0.21 ± 0.07 a
400 mM NaCl	B0	8.4 ± 1.6 gh	7.3 ± 1.5 fg	0.83 ± 0.14 de	0.12 ± 0.01 fg	0.14 ± 0.01 fg
	R	14.5 ± 2.3 abc	10.3 ± 0.8 bcde	0.98 ± 0.12 d	0.17 ± 0.02 bc	0.19 ± 0.01 b
	E	11.3 ± 1.0 cdefg	12.3 ± 2.0 ab	1.23 ± 0.08 c	0.15 ± 0.00 d	0.18 ± 0.02 b
	R + E	11.7 ± 2.6 cdefg	13.7 ± 1.1 a	1.15 ± 0.04 c	0.13 ± 0.01 ef	0.17 ± 0.03 cd
600 mM NaCl	B0	7.4 ± 0.2 h	5.3 ± 1.5 g	0.82 ± 0.01 de	0.11 ± 0.00 g	0.13 ± 0.00 g
	R	13.7 ± 1.9 bcd	9.0 ± 1.7 def	0.90 ± 0.07 de	0.14 ± 0.00 e	0.17 ± 0.01 cd
	E	9.8 ± 1.5 efgh	11.0 ± 1.7 abcd	0.81 ± 0.05 de	0.13 ± 0.01 ef	0.16 ± 0.01 de
	R + E	11.8 ± 1.4 cdef	7.7 ± 0.6 efg	0.82 ± 0.06 de	0.14 ± 0.01 e	0.17 ± 0.01 c
Sources of variation	*df*	Plant height	Root length	Shoot dry weight	Root dry weight	Root dry weight/shoot dry weight
Salinity levels (S)	3	**	**	**	**	**
Bacterial strains (B)	3	**	**	**	**	**
S × B	9	**	**	**	**	**
Error	32					
CV (%)		13.9	14.9	8.9	6.0	2.6

B0, control; R, rhizosphere strain *Staphylococcus* sp. R11; E, endophytic strain *Staphylococcus* sp. E14; CV, coefficient of variation; and **, Significant at $P < 0.01$. Data represent the mean ± SD (n = 3) and different letters indicate a significant difference ($P < 0.05$) using Tukey's test.

non–inoculated control (Table 1).

With increasing the concentration of NaCl up to 400 Mm NaCl, in the non–bacterial treatments, the amount of shoot dry weight increased and its maximum value was obtained at 200 mM NaCl concentration (0.9 ± 0.1 g pot^{-1}), but again its value dropped at 600 mM NaCl concentration (Table 1). Under salinity stress conditions, bacterial isolates also increased the shoot dry weight by a concentration of 400 mM NaCl. Under salinity stress, the highest amount of shoot dry weight (2.2 ± 0.2 g pot^{-1}) was recorded in plants co–inoculation with bacterial strains at a concentration of 200 mM NaCl.

With increasing the concentration of NaCl in non–inoculated conditions (B0) to 200 mM NaCl, the root dry weight increased by 69.7%. However, with increasing the NaCl concentration from 200 to 400 and 600 mM NaCl, root dry weight decreased to 35.6 and 12.7%, respectively, compared to the root dry weight of the plant at a concentration of 200 mM NaCl (Table 1). Inoculation of bacterial strains had a significant effect on root dry weight at 0 mM NaCl relative to control (B0). The highest amount of root dry weight (0.2 ± 0.0) was obtained from the plants co–inoculated with bacterial strains at 200 mM NaCl concentration. Similar to the shoot dry weight, root dry weight also decreased at salinity of 600 mM NaCl.

At 0 mM NaCl concentration, the highest amount root dry weight to shoot dry weight ratio was obtained in the treatment of co–inoculation with rhizosphere and endophytic isolates (Table 1). With increasing NaCl concentration from 0 to 200 mM NaCl, this ratio increased. This increase for non–bacterial treatment (B0), rhizosphere bacterial treatment (R), endophytic bacterial treatment (E), and co–inoculation treatment (R + E) was 7.0, 9.0, 26.7, and 18.2%, respectively. However, with increasing NaCl concentration from 200 to 400 and 600 mM NaCl, this ratio showed a decreasing trend.

In general, the co–inoculation of the halophyte *Salicornia* sp. with rhizosphere and endophytic bacterial strains plus 200 mM NaCl showed the best result in all measured morphological parameters (Fig. 3) except for root length, which showed the height amount in the *Salicornia* sp. co–inoculated with rhizosphere and endophytic bacterial strains plus 400 mM NaCl.

3.8. The effect of strains E14 and R11on plant physiological parameters

The leaf relative water content (RWC) increased significantly ($P < 0.05$) with increasing salinity levels. With increasing the concentration of NaCl from 0 to 200 mM NaCl, the leaf RWC in all treatments increased (Table 2). The lowest (5.4%) and the highest (16.1%) increase in leaf RWC were observed in the plants co–inoculated with rhizosphere and endophytic strains and non–inoculated plants (B0). In general, the effect of bacterial treatments on leaf RWC was different so that at salinity levels 0, 200, and 400 mM NaCl, strains R and E increased leaf RWC in comparison with the control (B0) and at salinity level 600 mM NaCl, these strains led to a decrease in leaf RWC compared to the control (Table 2).

A gradual decline in total chlorophyll content of plant with an increase in NaCl concentration was observed. However, the total chlorophyll content of plants inoculated with bacterial strains was higher than plants non–inoculated with these bacterial strains (control plants). The plants co–inoculated with bacterial strains and treated with 200 mM NaCl registered higher values (Table 2).

At the concentration of 0 mM NaCl, the effect of inoculation of bacterial strains on amount of the shoot K^+ was significant ($P < 0.05$) compared to control (Table 2). The highest amount of the shoot K^+ was obtained in plants inoculated with rhizosphere strain (17.8% increase compared to control) at the concentration of 0 mM NaCl. With increasing the concentration of NaCl to 200 and 400 mM NaCl, the effect of the bacterial strains on the shoot K^+ content was changed, as the highest shoot K^+ content was registered in the plants co–inoculated with the rhizosphere and endophytic strains with 22.8 and 21.9% increase compared to control under 200 and 400 mM NaCl concentration, respectively. However, with increasing concentration of NaCl from 400 to 600 mM NaCl, the effective amount of bacterial strains on shoot K^+ content was decreased, but still the highest shoot K^+ content (86.6% increase compared to the control) was obtained by co–inoculation of the plant with bacterial strains (Table 2).

Compared with the control group, salinity stress increased superoxide dismutase (SOD) activity. However, inoculation of the plants with bacterial strains significantly ($P < 0.05$) increased activity of the antioxidant enzyme compared with non–inoculated plants (Table 2). With increasing the NaCl concentration from 200 mM NaCl to 400 and

Fig. 3. Photos of *Salicornia* sp. plants treated with bacterial strains under various salinity levels after 80 days of sowing under greenhouse conditions. B, no bacteria; R, rhizosphere strain *Staphylococcus* sp. R11; E, endophytic strain *Staphylococcus* sp. E14; R + E, Staphylococcus sp. R11 + *Staphylococcus* sp. E14; 0, 0 mM NaCl; 200, 200 mM NaCl; 400, 400 mM NaCl; and 600, 600 mM NaCl.

600 mM NaCl, the effect of the co–inoculation of the rhizosphere and endophytic strains increased, as at concentration of 600 mM NaCl, the highest enzymatic activity of SOD (39.5 ± 0.7 U mg^{-1} FW) was obtained by co–inoculation of rhizosphere and endophytic strains.

Proline content increased in the leaves of the *Salicornia* sp. plant with increasing NaCl concentration. However, inoculation of bacterial isolates decreased the content of proline in the leaves of this plant. Under all salinity levels, the lowest content of proline was observed in the leaves of plants co–inoculated with bacterial isolates (Table 2).

The effect of bacterial treatment and interaction of bacterial treatment and salinity treatment on K^+/Na^+ ratio and shoot Na^+ concentration were not significant, but the effect of salinity treatment on this ratio and shoot Na^+ concentration was significant at 1% level. With increasing the concentration of NaCl, shoot Na^+ concentration increased and as a result, shoot K^+/Na^+ ratio decreased. The lowest K^+/Na^+ ratio (a decrease of 62.5% compared to the concentration of 0 mM NaCl) registered at a concentration of 600 mM NaCl (Fig. 4A).

At all salinity levels, the highest STI was observed in plants inoculated with bacterial strains (Fig. 4B). At salinity levels 200, 400 and 600 mM NaCl, STI in plants co–inoculated with the bacterial strains (R + E), in plants inoculated with endophytic strain (E), and in plants inoculated with rhizosphere strain (E) was the highest, respectively.

4. Discussion

The soil salinity is a major agricultural problem frequently prevailing in arid and semi–arid soils all around the world. Although salts are required by plants in certain amounts to maintain normal functions, but their high concentration in the soil inhibits non–halophytic plant metabolic functions. In addition to non–halophytic plants, it is known that the initial establishment of seeds of halophytic plants is delayed under conditions of high salt stress (Song et al., 2008). The association of plants and rhizosphere and endophytic bacteria are considered of great significance tolerance against environmental stresses (Etesami and Maheshwari, 2018). In addition, it has been proven that these habitat–adapted symbiotic rhizosphere and endophytic bacteria can have profound effects on host plant stress tolerance and fitness (Zhao et al., 2016).

Table 2
Mutual effects of treatments (levels of salinity and bacterial strains) on physiological parameters of the halophyte *Salicornia* sp. The plants were cultivated for 100 days in potted soil under greenhouse conditions.

Treatments		RWC (%)	Total chlorophyll (mg g^{-1} FW)	K^+ (%)	Superoxide dismutase activity (U mg^{-1} FW)	Proline (μmoL g^{-1} FW)
0 mM NaCl	B0	64.7 ± 1.4 g	1.20 ± 0.05 b	6.5 ± 0.4 defg	12.2 ± 1.8 i	0.15 ± 0.01 h
	R	67.9 ± 2.7 fg	1.25 ± 0.05 b	7.7 ± 0.6 ab	13.2 ± 1.8 i	0.17 ± 0.03 gh
	E	71.7 ± 1.9 ef	1.25 ± 0.04 b	6.9 ± 0.7 bcde	14.2 ± 1.0 i	0.16 ± 0.01 h
	R + E	68.3 ± 0.3 fg	1.31 ± 0.03 a	7.4 ± 0.4 abc	12.4 ± 1.0 i	0.16 ± 0.02 h
200 mM NaCl	B0	75.2 ± 3.4 de	0.81 ± 0.05 f	6.6 ± 0.4 def	16.8 ± 1.30 h	0.38 ± 0.02 e
	R	77.5 ± 0.4 cd	0.97 ± 0.04 d	7.6 ± 0.5 abc	19.4 ± 0.9 g	0.25 ± 0.06 f
	E	77.3 ± 9.0 cde	1.00 ± 0.06 d	7.7 ± 0.4 ab	21.6 ± 1.0 fg	0.24 ± 0.03 f
	R + E	72.0 ± 1.8 def	1.32 ± 0.05 a	8.0 ± 0.2 a	21.0 ± 2.6 g	0.22 ± 0.02 fg
400 mM NaCl	B0	73.2 ± 0.9 def	0.65 ± 0.02 g	5.9 ± 0.4 fghi	23.5 ± 2.2 ef	1.14 ± 0.04 b
	R	81.3 ± 3.6 c	0.81 ± 0.03 f	6.9 ± 0.7 cde	25.6 ± 1.2 e	0.92 ± 0.07 c
	E	75.9 ± 2.0 de	0.80 ± 0.04 f	7.0 ± 0.2 bcde	28.4 ± 0.4 d	0.84 ± 0.04 d
	R + E	72.2 ± 1.0 def	1.09 ± 0.03 c	7.2 ± 0.1 bcd	28.3 ± 1.3 d	0.83 ± 0.01 d
600 mM NaCl	B0	99.9 ± 1.0 a	0.51 ± 0.06 h	5.5 ± 0.2 i	31.0 ± 0.4 c	1.33 ± 0.02 a
	R	75.8 ± 4.4 de	0.63 ± 0.05 g	5.7 ± 0.4 hi	35.6 ± 0.8 b	1.30 ± 0.04 a
	E	86.6 ± 1.3 b	0.65 ± 0.02 g	5.6 ± 0.2 i	35.2 ± 1.0 b	1.31 ± 0.02 a
	R + E	82.4 ± 1.2 bc	0.86 ± 0.02 e	5.8 ± 0.3 ghi	39.5 ± 0.7 a	1.28 ± 0.03 a
Sources of variation	*df*	RWC	Total chlorophyll	K^+	Superoxide dismutase activity	Proline
Salinity levels (S)	3	**	**	**	**	**
Bacterial strains (B)	3	**	**	**	**	**
S × B	9	**	**	**	**	**
Error	32					
CV (%)		4.0	3.5	6.2	5.8	4.8

B0, control; R, rhizosphere strain *Staphylococcus* sp. R11; E, endophytic strain *Staphylococcus* sp. E14; CV, coefficient of variation; and **, Significant at $P < 0.01$. Data represent the mean ± SD (n = 3) and different letters indicate a significant difference ($P < 0.05$) using Tukey's test.

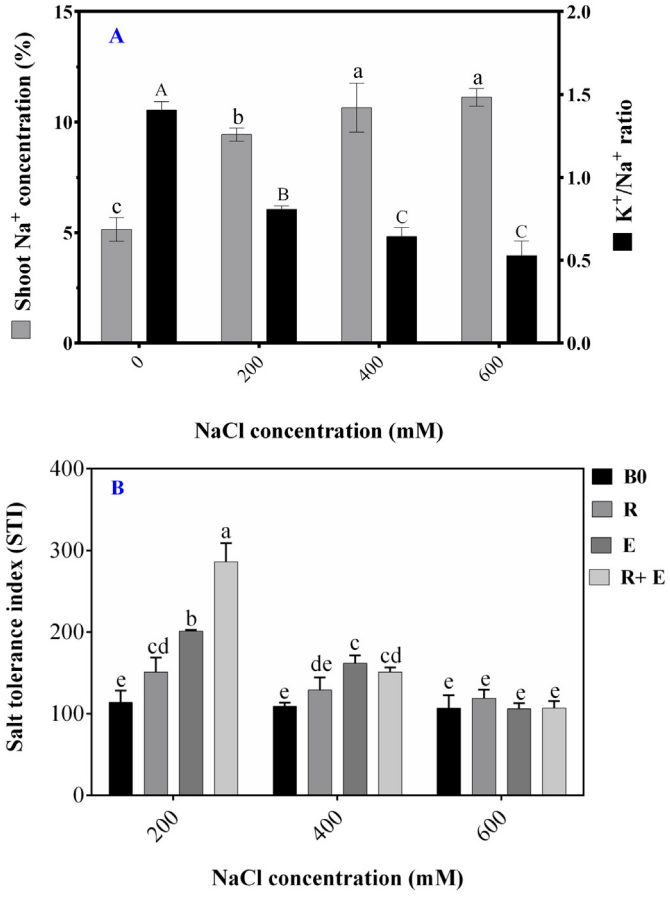

Fig. 4. Main effects of levels of salinity on K^+/Na^+ ratio and shoot Na^+ concentration of the halophyte *Salicornia* sp. (A) and salt tolerance index (STI) (B). The plants were cultivated for 100 days in potted soil under greenhouse conditions. B0, control; R, rhizosphere strain *Staphylococcus* sp. R11; and E, endophytic strain *Staphylococcus* sp. E14. Data represent the mean ± SD (n = 3) and different letters indicate a significant difference ($P < 0.05$) using Tukey's test.

The salt–accumulating halophyte *Salicornia* sp., frequently found in the salinized areas in of Iran, successfully adapts to saline environment and evolves different salt tolerance strategies. The optimal salinity for maximum growth of halophytes including *Salicornia* usually is in the range of 100–200 mM NaCl, and its growth is significantly hindered if the soil salinity is outside this range (Ma et al., 2013). In addition to various physiological and molecular level mechanisms, allowing the growth of halophytes in saline conditions (Flowers and Colmer, 2015), part of adaptive success of these plants may depend at least on their ability to establish and maintain effective associations with habitat–adapted rhizosphere and endophytic microorganisms (Etesami and Beattie, 2018; Zhao et al., 2016). In this study, we isolated endophytic and rhizosphere isolates associated with the halophyte *Salicornia* sp. and characterized them in terms of salinity and drought resistance and PGP traits. These bacterial isolates exhibited high salinity and drought tolerance. Compared to endophytic isolates, rhizospheric isolates were more resistant to high salinity. Since ionic strength is higher in rhizosphere, due to more water absorption, rhizospheric bacterial isolates are better adapted to high salinity. In previous research, salinity resistant–rhizospheric and endophytic bacteria were also isolated from halophytic plants including the halophyte *Salicornia* (Etesami and Beattie, 2018). For example, Zhao et al. (2016) tested the NaCl tolerance of 32 bacterial isolates isolated from *S. europaea*. Their results showed that all of which were able to tolerate 0.17 M NaCl. Rhizoshere and endophytic isolates selected in this study had insoluble inorganic phosphate–solubilizing abilities, which are considered as a possible mechanism by which these bacteria promote plant growth (Etesami and Maheshwari, 2018). All rhizoshere and endophytic isolates were able to produce IAA which can stimulate seed germination and augment the rate of root development (Etesami et al., 2015; Glick, 2012). The ability to produce this hormone as a secondary metabolite is reported in 80% of the bacteria associated with the plants (Olanrewaju et al., 2017). Compared to other PGP traits, fewer isolates had the ability to produce enzymes ACC–deaminase, which cleaves the ethylene precursor ACC. This enzyme can help seedlings or plants to withstand stress (biotic and abiotic) by reducing the level of “stress ethylene” (Glick, 2014). The ability to produce IAA and ACC deaminase and phosphate solubilization has been reported in bacteria isolated from various halophytes (Etesami and Beattie, 2018). Endophytic bacteria isolated from the

halophyte *S. europaea* by Zhao et al. (2016) had also the ability to produce IAA and ACC deaminase and solubilize inorganic phosphate.

In the present study, two effective endophytic and rhizospheric isolates were identified as *Staphylococcus* bacteria, which were physiologically characterized as beneficial and salt–tolerant under *in–vitro* conditions and they promoted *Salicornia* sp. growth by improving morphological and physiological traits of the plant. The *Staphylococcus* strains were also isolated from *Kallar* grass, which is a well-known halophytic plant and wildly found on saline areas (Ola et al., 2012). A number of *Staphylococcus* bacteria could tolerate high salt concentrations (Akram et al., 2016; Khan et al., 2015) and also had PGP traits (Akram et al., 2016; Zhou et al., 2015). Akram et al. (2016) reported the increased growth of maize plants in response to the inoculation of *Staphylococcus sciuri* SAT–17 under induced salinity stress.

It has been documented that inoculation of salt–tolerant beneficial bacteria in salt–affected soils has ultimately resulted in an increased plant biomass in addition to alleviation of deleterious effects of salt stress (Akram et al., 2016; Etesami and Beattie, 2018). Results of this study revealed that the inoculation of *Staphylococcus* strains R11, E14 and mixed inoculation significantly increased the *Salicornia* sp. growth under salinity stress. Similar improvement of plant growth by bacteria has been reported with other halophytes (Jha et al., 2012; Zhao et al., 2016). This growth–stimulating effect (i.e., increase in plant height, root length, and root and shoot dry weight) might be due to nutrient mobilization and IAA and ACC deaminase synthesis abilities. Both IAA and ACC deaminase–synthesis activities of PGPB are directly involved in plant growth and stress alleviation (Glick, 2014). The phyto–-hormone IAA might have stimulated the formation of root hairs followed by the intense colonization of plant with bacterial strains and more access of roots to nutrients and water (Etesami and Alikhani, 2016; Etesami et al., 2015). The results of Singh et al. (2015) also showed that inoculation of wheat plant with salinity resistant–PGPB resulted in better growth of the plant under 100 mM NaCl salinity stress conditions compared with plants non–inoculated with bacteria that attributed this effect to ACC deaminase activity.

The highest effect of bacterial isolates on *Salicornia* sp. growth indices was obtained at 200 mM NaCl concentration. Na^+ is an essential element for the growth of the halophytes including *Salicornia*, because halophytes need considerably larger amounts of Na^+, which is used as osmotic adjustment substance, for their growth (Flowers and Colmer, 2015). Thus, *Salicornia* sp. may grow well in high salt solution (200 mM NaCl) than in low salt solution (Na^+ deficiency). Similar result has been obtained previously for euhalophyte *S. brachiate* (Jha et al., 2012) and euhalophyte *S. europaea* (Zhao et al., 2016). Moreover, decrease in *Salicornia* sp. length and biomass with increasing salt stress (400 and 600 mM NaCl) may be attributed to limited water uptake due to the ion osmotic potential, reduced photosynthetic activity, less nutrient uptake and disturbance in many other metabolic functions (Shrivastava and Kumar, 2015). In this study, at a concentration of 600 mM NaCl, there was no significant difference in the shoot dry weight between the different levels of the bacteria. The probable reason is that these bacteria in the high salinity stress lost their PGP traits and were only able to survive in these conditions (Szymańska et al., 2018). It is noteworthy that these strains could also colonize plant roots and can be re–isolated from the host plant interior tissues and root surface under different salinity levels (data not shown). According to Hasanuzzaman et al. (2013), the cause of reducing the biomass of plants exposed to salinity is the limited supply of metabolites to plant growing–tissues due to decreasing the capacity of water absorption of root systems or increasing excessive absorption of Na^+ and Cl^- ions, which may cause significant physiological disturbances.

In the present study, the growth of the *Salicornia* sp. co–inoculated with endophytic and rhizosphere strains was more improved compared to the non–inoculated plant and plants inoculated with these strains separately under salt stress. In a previous study, the combined use of rhizosphere and endophytic bacteria in comparison with their separate use led to the best effect in increasing plant growth (Etesami and Alikhani, 2016). The maximum PGP potential of mixed inoculation may be attributed to commulative effect of these strains. It is well known that inoculation of plants with habitat–adapted rhizosphere and endophytic bacteria can be led to the significant enhancement in plant growth, yield and production under saline stress conditions (Etesami and Maheshwari, 2018) by various mechanisms. Salinity stress reduces osmotic pressure in the plant growth medium and diminishes water availability for the plant. In this study, inoculation of plant with salinity tolerant–PGP bacteria caused a decrease in the percentage of leaf RWC in salinity stress conditions, and the least percentage of leaf RWC was obtained from plants co–inoculated with bacterial strains. The reason for the decrease in the amount of water in tissues of inoculated plants is to reduce the amount of sodium accumulation in plant by bacteria (Ali et al., 2014) and reduce the need for water accumulation or root development, causing the improvement of the water–use efficiency of the plant (Mayak et al., 2004).

One of the indicators of salt stress–tolerance in plants is the increase in chlorophyll content. In this study, inoculation of plant with bacterial strains increased the amount of chlorophyll compared to control, but this difference was not significant. Singh and Jha (2017) also reported a decrease in plant chlorophyll content with increasing NaCl concentration. On the other hand, inoculation of the plant with beneficial bacteria increased the plant chlorophyll content in their study.

Salinity stress disrupts the balance of ions in plant cytosol. In this situation, the response and strategy of the plant to salinity is to exit Na^+ from the plant and enter K^+ to the plant. In contrast to sodium, increasing the concentration of K^+ can reduce the harmful effect of salinity on growth and yield (Shukla et al., 2012). In the present study, it was also found that the uptake of K^+ in *Salicornia* sp. showed decreasing trend with increasing NaCl concentration, probably due to increased uptake of Na^+ ions (Fig. 4A). It is well–established that under salt stress, the uptake of K^+ is reduced due to the antagonistic effect of Na^+ and K^+ (Rahneshan et al., 2018). The results of this study showed that inoculation of *Salicornia* sp. with bacterial strains reduced the sodium content, increased potassium, and increased potassium to sodium ratio in plant aerial parts. In all bacterial treatments, the potassium content was higher and the highest amount of potassium was observed in plants inoculated with the combination of rhizosphere and endophytic isolates. Singh and Jha (2017) also reported similar results with other plants. Increased uptake of K^+ as result of the inoculation with *Staphylococcus* strains R11, E14, and combined inoculum may be attributed to the plant growth–promoting and nutrient mobilization potential of the strains, root proliferation due to IAA production and low Na^+ uptake. In addition, by using Na^+/H^+ antiporters and increasing the activity of K^+/Na^+ transporters, salinity resistant–beneficial bacteria cause sodium depletion and increase potassium entry into the cell (Rojas-Tapias et al., 2012). K^+/Na^+ ratio was also found comparatively high in inoculated plants. The enhanced nutrient uptake may be due to rhizospheric competence and rhizospheric acidosis induced by PGPR inoculation (Shahid et al., 2012). However, the mechanisms underlying bacterially–induced ion regulation and nutrient translocation from soil to plant shoot are not completely understood. Furthermore, IAA production might have stimulated the uptake of K^+ with subsequent restriction in the uptake of Na^+ and this relation between ion regulation and nutrient uptake was reported in a previous study (Forni et al., 2017).

It is well–known that under the stress of salinity, reactive oxygen species (ROS) increase, so increasing oxidative stress reduces the amount of protein due to damage caused by oxidative conditions. One of the most important antioxidant mechanisms is the activity of the enzyme SOD (Sapre et al., 2018). It has been already established that bacteria induce stress tolerance in plants by triggering the cellular antioxidant levels (Shahid et al., 2018). In this study, with increasing NaCl concentration, the activity of the enzyme SOD increased, but this increase was higher in plants inoculated with bacterial strains than the

control. The highest activity of this enzyme was observed in the plants co–inoculated with rhizosphere and endophytic isolates at concentration of 600 mM NaCl (Table 2).

Proline is a very important osmolyte involved in osmoregulation and stabilization of many other macromolecules (Curá et al., 2017) and also is an important biochemical marker in the tolerance level of plants to salinity stress. Proline accumulation under salt stress plays a very important role in osmotic regulation. The results of this study showed that, with increasing the concentration of NaCl, the content of proline accumulation in the plant increased. However, inoculated plants showed reduced proline contents, which can be attributed to the fact that the inoculation of *Salicornia* sp. –plants with strains and combined inoculum alleviated the plants from salinity stress conditions. Sapre et al. (2018) also reported similar results in the effects of PGPB on proline accumulation in salinity stressed plants.

In general, the results of the present study agree with previous findings that halophytes are useful sources of halotolerant bacteria with the PGP potential. The results presented here also support the hypothesis that habitat–adapted rhizosphere and endophytic bacteria can contribute to the salt habitats adaptation of halophytes. Remarkably, the two promising strains, *Staphylococcus* sp. R11 and *Staphylococcus* sp. E14, displayed multiple traits, suggesting that the greater enhancement of growth of the halophyte *Salicornia* sp. may be attributable to a combination of the traits of these bacterial strains present in the inocula.

5. Conclusions

The results of the present study suggest that the halophyte *Salicornia* sp. naturally harbors a variety of putative endophytic and rhizosphere bacteria which exhibit tolerance to NaCl and drought and display different PGP traits. According to these results, the growth of *Salicornia* sp., as a halophytic plant, initially increased with increasing salinity and peaked at 200 mM NaCl. But its growth was reduced at higher salt concentrations (400 and 600 mM NaCl). However, inoculation of *Salicornia* sp. plant with effective salinity–resistant rhizosphere and endophytic strains resulted in the greater promotion of growth of the plant under saline conditions. The greatest effect of the isolates on plant growth indices was observed at 200 mM NaCl concentration, which could be due to a combination of the maximum effect of these bacteria on the plant growth and supply of the salt needed for the halophytic plant. In general, the results of this research show that *Salicornia* sp. plant has better performance in the presence of salinity–resistant PGP bacteria and, with less energy, can overcome the stress caused by high salt concentration. However, the inoculation experiments have to be extended to include repetitive field experiments for realizing the full potential of plant growth promotion of these strains. In addition, these bacteria probably have a potential in protecting other important plants against salt stress, which needs to be studied in the future.

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Author contributions

Contribution of the authors to this study was the same.

Conflict of interest

The authors have no conflict of interest.

References

Akram, M.S., Shahid, M., Tariq, M., Azeem, M., Javed, M.T., Saleem, S., Riaz, S., 2016. Deciphering Staphylococcus sciuri SAT-17 mediated anti-oxidative defense mechanisms and growth modulations in salt stressed maize (*Zea mays* L.). Front. Microbiol. 7, 867. https://doi.org/10.3389/fmicb.2016.00867.

Ali, S., Charles, T.C., Glick, B.R., 2014. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol. Biochem. 80, 160–167. https://doi.org/10.1016/j.plaphy.2014.04.003.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1. https://doi.org/10.1104/pp.24.1.1.

Bashan, Y., Holguin, G., 1997. *Azospirillum* – plant relationships: environmental and physiological advances (1990–1996). Can. J. Microbiol. 43, 103–121. https://doi.org/10.1139/m97-015.

Bashan, Y., Kamnev, A.A., de-Bashan, L.E., 2013. A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth. Biol. Fertil. Soils 1–2. https://doi.org/10.1007/s00374-012-0756-4.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207. https://doi.org/10.1007/BF00018060.

Curá, J.A., Franz, D.R., Filosofía, J.E., Balestrasse, K.B., Burgueño, L.E., 2017. Inoculation with *Azospirillum* sp. and *Herbaspirillum* sp. bacteria increases the tolerance of maize to drought stress. Microorganisms 5, 41. https://doi.org/10.3390/microorganisms5030041.

Davy, A.J., Bishop, G.F., Costa, C.S.B., 2001. *Salicornia* L. (*Salicornia pusilla* J. woods, S. ramosissima J. woods, S. europaea L., S. obscura PW ball & tutin, S. nitens PW ball & tutin, S. fragilis PW ball & tutin and S. dolichostachya moss). J. Ecol. 89, 681–707. https://doi.org/10.1046/j.0022-0477.2001.00607.x.

Dworkin, M., Foster, J.W., 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol. 75, 592.

Edwards, U., Rogall, T., Blocker, H., Emde, M., Bottger, E.C., 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res. 17, 7843–7853.

El Shaer, H.M., 2010. Halophytes and salt-tolerant plants as potential forage for ruminants in the Near East region. Small Rumin. Res. 91, 3–12. https://doi.org/10.1016/j.smallrumres.2010.01.010.

Etesami, H., Alikhani, H.A., 2016. Co-inoculation with endophytic and rhizosphere bacteria allows reduced application rates of N-fertilizer for rice plant. Rhizosphere. 2, 5–12. https://doi.org/10.1016/j.rhisph.2016.09.003.

Etesami, H., Beattie, G., 2018. Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. Front. Microbiol. 9, 148. https://doi.org/10.3389/fmicb.2018.00148.

Etesami, H., Maheshwari, D.K., 2018. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. Ecotoxicol. Environ. Saf. 156, 225–246. https://doi.org/10.1016/j.ecoenv.2018.03.013.

Etesami, H., Hosseini, H.M., Alikhani, H.A., Mohammadi, L., 2014. Bacterial biosynthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase and indole-3-acetic acid (IAA) as endophytic preferential selection traits by rice plant seedlings. J. Plant Growth Regul. 33, 654–670. https://doi.org/10.1007/s00344-014-9415-3.

Etesami, H., Alikhani, H.A., Hosseini, H.M., 2015. Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. MethodsX. 2, 72–78. https://doi.org/10.1016/j.mex.2015.02.008.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179, 945–963. https://doi.org/10.1111/j.1469-8137.2008.02531.x.

Flowers, T.J., Colmer, T.D., 2015. Plant salt tolerance: adaptations in halophytes. Ann. Bot. 115, 327–331. https://doi.org/10.1093/aob/mcu267.

Flowers, T.J., Galal, H.K., Bromham, L., 2010. Evolution of halophytes: multiple origins of salt tolerance in land plants. Funct. Plant Biol. 37, 604–612. https://doi.org/10.1071/FP09269.

Forni, C., Duca, D., Glick, B.R., 2017. Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil 410, 335–356. https://doi.org/10.1007/s11104-016-3007-x.

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases I. Occurrence in higher plants. Plant Physiol. 59, 309–314. https://doi.org/10.1104/pp.59.2.309.

Glenn, E.P., O'Leary, J.W., Watson, M.C., Thompson, T.L., Kuehl, R.O., 1991. *Salicornia bigelovii* Torr.: an oilseed halophyte for seawater irrigation. Science 251, 1065–1068. https://doi.org/10.1126/science.251.4997.1065.

Glick, B.R., 2012. Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012, 963401. https://doi.org/10.6064/2012/963401.

Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol. Res. 169, 30–39. https://doi.org/10.1016/j.micres.2013.09.009.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F., Kloepper, J.W., 1997. Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43, 895–914. https://doi.org/10.1139/m97-131.

Hasanuzzaman, M., Nahar, K., Fujita, M., 2013. Plant Response to Salt Stress and Role of Exogenous Protectants to Mitigate Salt-induced Damages, Ecophysiology and Responses of Plants Under Salt Stress. Springer, pp. 25–87. https://doi.org/10.1007/978-1-4614-4747-4_2.

Hasanuzzaman, M., Nahar, K., Alam, M.M., Bhowmik, P.C., Hossain, M.A., Rahman, M.M., Prasad, M.N.V., Ozturk, M., Fujita, M., 2014. Potential use of halophytes to remediate saline soils. Biomed. Res. Int. 2014. https://doi.org/10.1155/2014/589341.

Hiscox, J.D., Israelstam, G.F., 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57, 1332–1334. https://doi.org/10.1139/b79-163.

Jha, B., Gontia, I., Hartmann, A., 2012. The roots of the halophyte *Salicornia brachiata* are a source of new halotolerant diazotrophic bacteria with plant growth-promoting

potential. Plant Soil 356, 265–277. https://doi.org/10.1007/s11104-011-0877-9.
Khan, M.U., Sessitsch, A., Harris, M., Fatima, K., Imran, A., Arslan, M., Shabir, G., Khan, Q.M., Afzal, M., 2015. Cr-resistant rhizo-and endophytic bacteria associated with *Prosopis juliflora* and their potential as phytoremediation enhancing agents in metal-degraded soils. Front. Plant Sci. 5, 755. https://doi.org/10.3389/fpls.2014.00755.
Kohler, J., Hernández, J.A., Caravaca, F., Roldán, A., 2009. Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. Environ. Exp. Bot. 65, 245–252. https://doi.org/10.1016/j.envexpbot.2008.09.008.
Larsen, H., 1986. Halophilic and halotolerant microorganisms-an overview and historical perspective. FEMS Microbiol. Lett. 39, 3–7. https://doi.org/10.1111/j.1574-6968.1986.tb01835.x.
Lodewyckx, C., Vangronsveld, J., Porteous, F., Moore, E.R.B., Taghavi, S., Mezgeay, M., der Lelie, D.v., 2002. Endophytic bacteria and their potential applications. RC Crit. Rev. Plant Sci. 21, 583–606. https://doi.org/10.1080/0735-260291044377.
Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. Annu. Rev. Microbiol. 63, 541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918.
Ma, J., Zhang, M., Xiao, X., You, J., Wang, J., Wang, T., Yao, Y., Tian, C., 2013. Global transcriptome profiling of *Salicornia europaea* L. shoots under NaCl treatment. PLoS One 8, e65877. https://doi.org/10.1371/journal.pone.0065877. (Print 2013).
Mapelli, F., Marasco, R., Rolli, E., Barbato, M., Cherif, H., Guesmi, A., Ouzari, I., Daffonchio, D., Borin, S., 2013. Potential for plant growth promotion of rhizobacteria associated with *Salicornia* growing in Tunisian hypersaline soils. Biomed. Res. Int. 2013. https://doi.org/10.1155/2013/248078.
Marasco, R., Mapelli, F., Rolli, E., Mosqueira, M.J., Fusi, M., Bariselli, P., Reddy, M., Cherif, A., Tsiamis, G., Borin, S., 2016. Salicornia strobilacea (synonym of *Halocnemum strobilaceum*) grown under different tidal regimes selects rhizosphere bacteria capable of promoting plant growth. Front. Microbiol. 7, 1286. https://doi.org/10.3389/fmicb.2016.01286.
Mayak, S., Tirosh, T., Glick, B.R., 2004. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci. 166, 525–530. https://doi.org/10.1016/j.plantsci.2003.10.025.
Michel, B.E., Kaufmann, M.R., 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol. 51, 914–916. https://doi.org/10.1104/pp.51.5.914.
Okalebo, J.R., Gathua, K.W., Woomer, P.L., 2002. Laboratory Methods of Soil and Plant Analysis: A Working Manual, second edition. Sacred Africa, Nairobi.
Ola, H.A.E., Reham, E.F., Eisa, S.S., Habib, S.A., 2012. Morpho-anatomical changes in salt stressed *kallar* grass (*Leptochloa fusca* L. Kunth). Res. J. Agric. Biol. Sci. 8, 158–166.
Olanrewaju, O.S., Glick, B.R., Babalola, O.O., 2017. Mechanisms of action of plant growth promoting bacteria. World J. Microbiol. Biotechnol. 33, 197. https://doi.org/10.1007/s11274-017-2364-9.
Patten, C.L., Glick, B.R., 1996. Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol. 42, 207–220. https://doi.org/10.1139/m96-032.
Penrose, D.M., Glick, B.R., 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol. Plant. 118, 10–15. https://doi.org/10.1034/j.1399-3054.2003.00086.x.
Pillay, V.K., Nowak, J., 1997. Inoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. Can. J. Microbiol. 43 (4), 354–361. https://doi.org/10.1139/m97-049.
Qin, Y., Druzhinina, I.S., Pan, X., Yuan, Z., 2016. Microbially mediated plant salt tolerance and microbiome-based solutions for saline agriculture. Biotechnol. Adv. 34, 1245–1259. https://doi.org/10.1016/j.biotechadv.2016.08.005.
Rahneshan, Z., Nasibi, F., Moghadam, A.A., 2018. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. J. Plant Interact. 13, 73–82. https://doi.org/10.1080/17429145.2018.1424355.
Rodriguez, R.J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., Kim, Y.-O., Redman, R.S., 2008. Stress tolerance in plants via habitat-adapted symbiosis. ISME J. 2, 404. https://doi.org/10.1038/ismej.2007.106.
Rojas-Tapias, D., Moreno-Galván, A., Pardo-Díaz, S., Obando, M., Rivera, D., Bonilla, R., 2012. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). Appl. Soil Ecol. 61, 264–272. https://doi.org/10.1016/j.apsoil.2012.01.006.
Ryan, J., Estefan, G., Rashid, A., 2007. Soil and Plant Analysis Laboratory Manual. ICARDA.
Sapre, S., Gontia-Mishra, I., Tiwari, S., 2018. *Klebsiella* sp. confers enhanced tolerance to salinity and plant growth promotion in oat seedlings (*Avena sativa*). Microbiol. Res. 206, 25–32. https://doi.org/10.1016/j.micres.2017.09.009.
Shahid, M., Hameed, S., Imran, A., Ali, S., van Elsas, J.D., 2012. Root colonization and growth promotion of sunflower (*Helianthus annuus* L.) by phosphate solubilizing *Enterobacter* sp. Fs-11. World J. Microbiol. Biotechnol. 28, 2749–2758. https://doi.org/10.1007/s11274-012-1086-2.
Shahid, M., Akram, M.S., Khan, M.A., Zubair, M., Shah, S.M., Ismail, M., Shabir, G., Basheer, S., Aslam, K., Tariq, M., 2018. A phytobeneficial strain *Planomicrobium* sp. MSSA-10 triggered oxidative stress responsive mechanisms and regulated the growth of pea plants under induced saline environment. J. Appl. Microbiol. 124, 1566–1579. https://doi.org/10.1111/jam.13732.
Shrivastava, P., Kumar, R., 2015. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J. Biol. Sci. 22, 123–131. https://doi.org/10.1016/j.sjbs.2014.12.001.
Shukla, P.S., Agarwal, P.K., Jha, B., 2012. Improved salinity tolerance of *Arachishy pogaea* (L.) by the interaction of halotolerant plant-growth-promoting rhizobacteria. J. Plant Growth Regul. 31, 195–206. https://doi.org/10.1007/s11356-018-2381-8.
Singh, R.P., Jha, P.N., 2017. The PGPR *Stenotrophomonas maltophilia* SBP-9 augments resistance against biotic and abiotic stress in wheat plants. Front. Microbiol. 8, 1945. https://doi.org/10.3389/fmicb.2017.01945.
Singh, R.P., Jha, P., Jha, P.N., 2015. The plant-growth-promoting bacterium *Klebsiella* sp. SBP-8 confers induced systemic tolerance in wheat (*Triticum aestivum*) under salt stress. J. Plant Physiol. 184, 57–67. https://doi.org/10.1016/j.jplph.2015.07.002.
Sobhanian, H., Aghaei, K., Komatsu, S., 2011. Changes in the plant proteome resulting from salt stress: toward the creation of salt-tolerant crops? J. Proteome 74, 1323–1337. https://doi.org/10.1016/j.jprot.2011.03.018.
Song, J., Fan, H., Zhao, Y., Jia, Y., Du, X., Wang, B., 2008. Effect of salinity on germination, seedling emergence, seedling growth and ion accumulation of a euhalophyte *Suaeda salsa* in an intertidal zone and on saline inland. Aquat. Bot. 88, 331–337. https://doi.org/10.1016/j.aquabot.2007.11.004.
Sperber, J.I., 1958. Solution of apatite by soil microorganisms producing organic acids. Crop Pasture Sci. 9, 782–787. https://doi.org/10.1071/AR9580782.
Sturz, A.V., Christie, B.R., Nowak, J., 2000. Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit. Rev. Plant Sci. 19, 1–30. https://doi.org/10.1016/S0735-2689(01)80001-0.
Szymańska, S., Płociniczak, T., Piotrowska-Seget, Z., Hrynkiewicz, K., 2016. Endophytic and rhizosphere bacteria associated with the roots of the halophyte *Salicornia europaea* L.–community structure and metabolic potential. Microbiol. Res. 192, 37–51. https://doi.org/10.1016/j.micres.2016.05.012.
Szymańska, S., Borruso, L., Brusetti, L., Hulisz, P., Furtado, B., Hrynkiewicz, K., 2018. Bacterial microbiome of root-associated endophytes of *Salicornia europaea* in correspondence to different levels of salinity. Environ. Sci. Pollut. Res. 25, 25420–25431. https://doi.org/10.1007/s11356-018-2530-0.
Weatherley, P., 1950. Studies in the water relations of the cotton plant: I. The field measurement of water deficits in leaves. New Phytol. 49, 81–97. https://doi.org/10.1111/j.1469-8137.1950.tb05146.x.
Yuan, X.K., Yang, Z.Q., Li, Y.X., Liu, Q., Han, W., 2016. Effects of different levels of water stress on leaf photosynthetic characteristics and antioxidant enzyme activities of greenhouse tomato. Photosynthetica. 54, 28–39. https://doi.org/10.1111/j.1744-7909.2006.00272.x.
Zhao, S., Zhou, N., Zhao, Z.-Y., Zhang, K., Wu, G.-H., Tian, C.-Y., 2016. Isolation of endophytic plant growth-promoting bacteria associated with the halophyte *Salicornia europaea* and evaluation of their promoting activity under salt stress. Curr. Microbiol. 73, 574–581. https://doi.org/10.1007/s00284-016-1096-7.
Zhou, W., Qin, S., Lyu, D., Zhang, P., 2015. Soil sterilisation and plant growth-promoting rhizobacteria promote root respiration and growth of sweet cherry rootstocks. Arch. Agron. Soil Sci. 61, 361–370. https://doi.org/10.1080/03650340.2014.935346.
Zhu, F., Qu, L., Hong, X., Sun, X., 2011. Isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the coast of Yellow Sea of China. Evid. Based Complement. Alternat. Med. 2011. https://doi.org/10.1155/2011/615032.