Journal of Plant Growth Regulation (2023) 42:946–959
https://doi.org/10.1007/s00344-022-10605-5

Extremophiles as Plant Probiotics to Promote Germination and Alleviate Salt Stress in Soybean

Ana P. Santos[1] · Carolina Belfiore[1] · Cristina Úrbez[2] · Alejandro Ferrando[2] · Miguel A. Blázquez[2] · María E. Farías[1]

Received: 2 April 2021 / Accepted: 7 February 2022 / Published online: 18 February 2022

Abstract

Bacteria isolated from extreme environments have been shown to promote plant growth under challenging conditions. This study aimed to examine the performance of the extremophilic microorganisms under salt stress and their ability to improve the tolerance of soybean plants to this stress. In vitro experiments showed that bacteria belonging to the genera *Stenotrophomonas* and *Exiguobacterium* were halophilic and displayed plant growth-promoting activities under salt stress. For instance, these two species enhanced soybean germination rate between 35 and 43% in comparison with non-inoculated seeds. In addition, inoculation allowed soybean roots to double their size, both in length and in dry biomass, under 250 mM NaCl. The plant physiological responses correlated with changes in plant gene expression during developmental and stress responses. The expression of a growth marker gene (*Glyma.03G226000*) increased in the presence of halophilic bacteria both under salt stress (5- to 24-fold) and under non-stress conditions (17- to 25-fold). Soybean genes responsive to stress, such as *Glyma.02G228100*, *Glyma.04G180400*, *Glyma.08G189600,* and *Glyma.17G173200,* were highly induced by salt in bacteria-inoculated roots. This work showed that the extremophilic bacteria used in these experiments could be used as potential bio-inoculants to help alleviate salt stress and plant growth.

Keywords Soybean · Seed germination · Salt stress · Extremophilic microorganisms

Introduction

Soil salinity affects an estimated 1125 million hectares worldwide (Hossain 2019). Particularly, Argentina is classified as the third country with the largest area of soil affected by salinity, after Russia and Australia (Puchulú 2008). Recent reports have stated that around 37 million hectares, including arid and semi-arid zones, are affected by an excess of salts and sodium in Argentina (Rodríguez et al. 2019). The desertification of soils and toxic salt accumulation represent major environmental concerns due to the severe need for food security associated with the increasing world population (Ilangumaran and Smith 2017). For this reason, researchers have attempted to develop several strategies to mitigate salt stress, but the increased labor intensiveness and the cost of the required methods have led to the search for alternatives such as the use of plant growth-promoting bacteria (PGPB) as a means to alleviate plant stress (Mayak et al. 2004).

PGPB have agricultural importance because they offer a wide range of benefits including the ability to increase plant growth, stimulate seed germination, improve seedling emergence by increasing the availability of nutrient absorption, and stimulate the production of phytohormones such as indole-3-acetic acids, gibberellins, and cytokinin (Harman and Björkman 1998; Lugtenberg et al. 2001). Extreme environments are potential sources of PGPB strains (Tapia-Vázquez et al. 2020) due to their extraordinary capacity of adaptation to adverse environmental conditions. Their survival strategies, including the ability to produce secondary metabolites and robust enzymes, make extremophilic microorganisms suitable for biotechnological purposes and useful

Handling Editor: Parvaiz Ahmad.

✉ María E. Farías
mefarias2009@gmail.com

[1] Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas (LIMLA), Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Avenida Belgrano y Pasaje Caseros, San Miguel de Tucumán, Argentina

[2] Instituto de Biología Molecular y Celular de Plantas (IBMCP), Consejo Superior de Investigaciones Científicas, Universitat Politècnica de València (CSIC-UPV), C/ Ingeniero Fausto Elio s/n, 46022 Valencia, Spain

in different agricultural practices (Yadav 2017). The mitigation of salt stress by these bio-inoculants has been shown in different crops such as rice (Rangarajan et al. 2003; Sapsirisopa et al. 2009; Nautiyal et al. 2013), wheat (Egamberdieva and Kucharova 2009), maize (Egamberdiyeva 2007), lettuce (Han and Lee 2005), tomato (Mayak et al. 2004), and pepper (Del-amor and Cuadra-Crespo 2012; Siddikee et al. 2011). Therefore, extremophiles represent a vast repertoire of biofertilizers; however, whether extremophilic microorganisms can improve productivity of crop plants such as soybean (*Glycine max* L.) has been little explored (García et al. 2021; Belfiore et al. 2018a, b).

Soybean is classified as moderately salt-tolerant, with salinity causing adverse effects at every developmental stage. Crop yield begins to decline when soil salinity exceeds 5 dS/m (i.e., 50 mM) (Ashraf 1994; Papiernik et al. 2005) by inhibiting seed germination and post-germinative growth. Soybean plants have developed several mechanisms to cope with salt stress and the availability of the whole soybean genome sequence improved our understanding of the basic mechanisms of how salinity affects gene expression and regulation (Khan et al. 2021). Considering that salt tolerance at the germination stage is important for soybean establishment in saline soils, and intolerance to salinity may result in physiological and biochemical disorders (Rehman et al. 1996; Ungar 1996), we have evaluated the interactive mechanism of extremophilic microorganisms with soybean during severe stress imposed by salinity. To this aim, bacterial isolates from extreme environments in the Andean soil, such as *Exiguobacterium* sp. S58, S56a, S60, and *Stenotrophomonas* sp. AG3 strains (Belfiore et al. 2018a, b), were selected since they had been previously reported as potential PGPB candidates for soybean under non-stress conditions (Belfiore et al. 2018a, b). In this study, the performance of these four strains under salt stress was analyzed by testing both the capacity of the bacteria to display PGP-related traits as well as their ability to improve soybean salt tolerance. Additionally, the expression of some candidate salt stress-responsive genes was evaluated in soybean plants after cocultivation with the PGPB.

Materials and Methods

Bacterial Strains and Inoculum Preparation

Four strains of *Stenotrophomonas* sp. AG3, *Exiguobacterium* sp. S56a, S58, and S60 isolated, identified (GenBank accession numbers: MW442973, MW442975, MW442976 and MW442974), and characterized as PGPB under normal conditions in previous work (Belfiore et al. 2018a, b) were used in this study. The preparation of bacterial strains was performed at the Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas (LIMLA) and the bacteria were deposited in PROIMI culture collection (Word Data Center for Microorganisms 587) with the strain numbers: PROIMI100153 (AG3), PROIMI100154 (S56a), PROIMI100152 (S60) and PROIMI100155 (S58). All bacterial strain cultures were grown in a nutrient broth medium (g/L composition: 3 meat extract, 5 triptein, 15 agar) at 30 °C overnight, 200 rpm and maintained in 50% glycerol stock at − 80 °C. The cultures were sub-cultured twice before use.

Screening for Salt-Tolerant Bacterial Strains

The tolerance of bacterial isolates to salinity was assessed by growing in nutrient broth medium with different concentrations of NaCl (0, 250, 500, 750, 1000, 1250, 1500 and 2500 mM). 100 µL of each culture aliquot was inoculated into 10 mL sterilized nutrient broth medium ($OD_{600\ nm} \approx 0.1$) and incubated at 30 °C, 200 rpm for 48 h. Growth performance was monitored by measuring the optical density at 600 nm with a UV–Vis spectrophotometer (Rayleigh) and using a colony counting technique on each saline nutrient agar medium (NA). The concentration of colony-forming units (CFU/mL) was expressed using the average of the plate counts from the experimental triplicates.

Characterization of Strains as PGPB Under Salt Stress

The experiments were performed in triplicate, in the presence of different concentrations of NaCl. The parameters evaluated were as follows.

Production of Indole Acetic Acid (IAA)

IAA production was determined by colorimetry using the Salkowski's reagent (Tang and Bonner 1948) with some modifications. The bacteria were grown in a modified nutrient broth medium with 1 mg/mL of tryptophan and NaCl (0, 250, 750 and 1500 mM), at 30 °C for 72 h to induce the production of IAA. After 72 h, the cultures were centrifuged at 5000 rpm for 15 min followed by removal and mixing of the supernatant with Salkowski's reagent. Optical density was measured by absorbance at 530 nm. The quantity of IAA produced by bacterial isolates was determined by comparison of absorbance values with those from a standard curve of pure IAA (Sigma-Aldrich Co.) in the range 0–60 µg/mL.

Phosphate Solubilization

The phosphate solubilization was analyzed with modified Sundara Rao Sinha medium (SRSM) with NaCl (0, 250, 750 and 1500 mM), according to the method described by Vázquez et al. (2000). The clear zone around the growth

area was used to calculate the phosphate solubilization index (PSI).

Siderophore Production

Siderophore production was determined by the method of Schwyn and Neilands (1987) on chromium azurol sulfonate agar (CAS) medium amended with 0, 250,750 and 1500 mM NaCl. The plates were incubated at 30 °C for 24 h, and the orange halo development around the growth indicated siderophore production.

Nitrogen-Fixing Activity

The bacterial cultures were inoculated in a semi-solid nitrogen-free medium (Nfb) with bromothymol blue (Dobereiner 1995) and NaCl (0, 250,750 and 1500 mM). Plates were incubated at 30 °C for 7 days; an enhanced blue color of the bromothymol blue around the colony qualitatively indicated the positive effect of nitrogen-fixing activity.

1-Aminocyclopropane-1-carboxylate (ACC) Deaminase Activity

ACC deaminase activity was quantitatively measured by the method of Penrose et al. (2003) determining the amount of α-ketobutyrate produced after the enzymatic cleavage of ACC. The ACC deaminase activity was determined at different salt (NaCl) concentrations ranging from 0 to 1500 mM.

Exopolysaccharide (EPS) Production

The EPS production was evaluated in an optimized medium reported by Verhoef et al. (2003) supplemented with different concentrations of NaCl (0, 250,750 and 1500 mM). 100 mL of medium were inoculated with 1 mL overnight bacteria culture ($OD_{600\ nm} \approx 0.1$) and incubated at 30 °C, 200 rpm for 48 h. EPS was extracted and quantified (g 100 mL^{-1} culture) by the method described in Kumari et al. 2015.

Evaluation of Biofilm Production

Biofilm Formation Assay

Bacterial isolates were tested for their ability to form biofilms in modified biofilm-promoting minimal medium M63 supplemented with 0.2% (w/v) glucose, 1 mM $MgSO_4$, 0.5% (w/v) casamino acids and different concentrations of NaCl (0, 250,750 and 1500 mM) according to O'Toole (2011) protocol. The biofilm formation was quantified by measuring the absorbance at 570 nm with Elisa Reader spectrophotometer (Multiskan Go, Termo scientific).

Biofilm Imaging with Scanning Electron Microscopy (SEM)

7 day-old soybean plants, grown under sterile conditions, were transplanted to culture tubes containing biofilm-promoting minimal medium M63 and different concentrations of NaCl (0 and 250 mM). The tubes were inoculated with each strain and incubated at 30 °C for 48 h. After the incubation, the plant roots were cut, rinsed with physiological solution, and fixed in Karnosvsky buffer (2.5% glutaraldehyde and 1.5% paraformaldehyde) pH 7.2, for 24 h. The samples were dehydrated successively with ethanol (30%, 50%, 70%, and 90%) for 10 min and finally maintained in acetone (100%). Complete dehydration of samples was carried out with the critical point technique and coated with gold by sputtering. The microscope visualization was performed using a ZEISS SUPRA 55 VP scanning from CISME (Centro de Investigaciones y Servicios de Microscopía Electrónica). Roots without inoculum were used as a control.

Bacterial Treatments and Soybean Germination Under NaCl Stress

Germination traits were examined at the Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas (LIMLA-CONICET), and soybean gene expression analysis was carried out at the Instituto de Biología Molecular y Celular de Plantas (IBMCP).

Inoculum Preparation

The selected strains were grown in a nutrient broth medium at 30 °C for 24 h. After incubation, the cells were centrifuged at 10,000 rpm for 15 min and washed with distilled sterile water in order to remove any culture medium residue. The bacterial suspension was adjusted to 10^8 CFU/mL using a spectrophotometer (Rayleigh) and OD 600 nm.

Seed Sterilization and Inoculation

Soybean seeds (BAYER Credenz 4505B RR1-STS) were sterilized with ethanol (70%) three times for 15 min, rinsed with distilled sterile water five times, and drained on a clean bench. The seeds were gently stirred with the corresponding bacterial suspensions (10^8 CFU/mL) for 5 min at room temperature. Sterile distilled water was used instead of bacterial suspension as a control.

For each treatment, germination was performed by sowing 20 seeds on top of water agar (1% w/v) Petri dishes under two conditions: water agar without salt and water agar with a final concentration of 250 mM NaCl. The plates were sealed with parafilm and placed in a germination chamber (no humidity control) for 7 days at 26 °C in the dark. After 7 days, roots were removed from the Petri dishes, and the

following parameters were evaluated: fresh and dry (105 °C overnight) root biomass (mg), length (cm), relative root surface area, and germination percentage.

The relative root surface area was estimated by the gravimetric method (Carley and Watson 1966). Air-dried roots were immersed in a Ca $(NO_3)_2$ saturated solution and the weight (mg) of Ca $(NO_3)_2$ removed from the solution was recorded.

For the germination percentage, the number of emerged seeds was scored every day and calculated as follows:

$$\text{Germination}\% = \left[(\text{Number of germinated seeds}/\text{total number of seeds tested}) \times 100\right]$$

The assay was conducted with three biological replicates.

Gene Expression Analysis by RTqPCR

After treatment, plant root samples were frozen with liquid nitrogen and stored at − 80 °C. Total RNA was extracted using the RNA Plant mini spin kit (Macherey–Nagel NucleoSpin®). cDNA was synthesized using the Prime Script ™ First-Strand Synthesis System for RT-PCR (Takara Bio) and analyzed on 7500 Fast Real-Time PCR System (Applied Biosystems) with SYBR Premix Ex Taq II (Tli RNaseH Plus) ROX plus (Takara Bio) according to the manufacturer's protocols. To normalize the gene expression, *Glyma* UKN1 was selected as a reference gene. Quantification of the relative gene expression levels was performed according to the $2^{\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Primer sequences are indicated in Table S1.

Statistical Analysis

To analyze the growth parameters, data were subjected to analysis of variance (ANOVA) using GraphPad Prism 7.0 software. Treatment means were separated by Tukey's HSD test, and the level of significance was set at $p < 0.05$.

Results

Influence of Salt on the Behavior of Selected Bacterial Strains

Organisms such as halotolerant bacteria that naturally live in extreme environments are referred to as extremophiles (Siddikee et al. 2011) and serve as a useful tool in sustainable agriculture for their potential to alleviate salt stress. The salt tolerance of four previously isolated strains (Belfiore et al 2018a, b)—*Stenotrophomonas* sp. AG3 and three *Exiguobacterium* species (sp. S58, S56a, and S60)—was determined by counting total colony number per milliliter in NA medium with increasing concentrations of NaCl (Fig. 1). The results showed that the bacterial isolates could stand the addition of 1250 mM NaCl to the NA

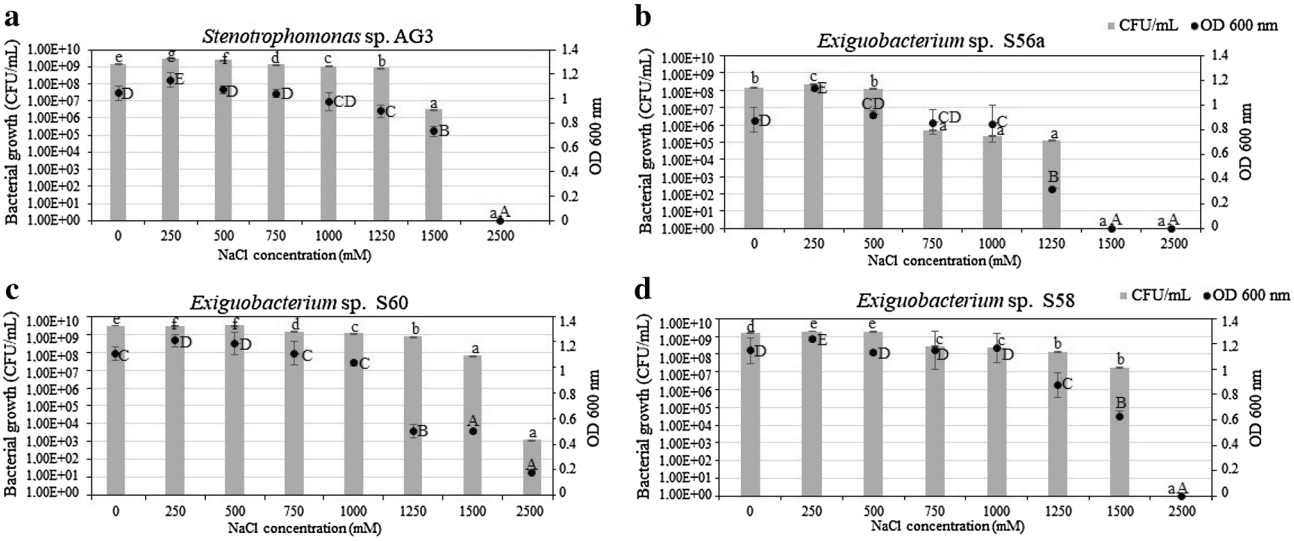


Fig. 1 The effect of different NaCl concentrations on bacterial growth after 48 h. Values represent the mean ± standard error ($n = 3$). Different letters on each bar indicate significant differences among means ($p < 0.05$) according to Tukey's HSD test. Lowercase letters on the bar compare the number of colony-forming units (CFU/mL) under different salt treatments. Uppercase letters compare the optical density measurements of bacteria under each salinity treatment

medium. Therefore, the four bacteria could be classified as moderately halophilic microorganisms (Edbeib et al. 2016). At a higher concentration of salt, i.e., 1500 mM NaCl, the growth rate started to decline for *Stenotrophomonas* sp. AG3, *Exiguobacterium* sp. S60 and S56a, while for *Exiguobacterium* sp. S58 growth was suppressed. The highest concentration of NaCl tested (2500 mM) prevented the growth of three of them, *Stenotrophomonas* sp. AG3, and *Exiguobacterium* sp. S58 and S56a, but not *Exiguobacterium* sp. S60.

Previous studies reported that halotolerant bacteria display growth up to 1300 mM of NaCl (Nakbanpote et al. 2014). In this study, we expanded and evaluated the PGP potential of the different bacteria under higher NaCl concentrations in a qualitative and quantitative manner. The bacterial strains were grown in media containing no-salt, 250, 750, and 1500 mM NaCl, and the following factors were determined: (i) IAA production, (ii) phosphate solubilization, (iii) siderophores production, (iv) nitrogen-fixing performance, (v) ACC deaminase activity, (vi) EPS production, and (vii) biofilm production (Table 1).

While the presence of either low or high salt concentrations did not affect the ability of *Stenotrophomonas* sp. AG3 and *Exiguobacterium* sp. S58 to solubilize phosphate (Table 1), other traits were altered by the presence of salt to different extents depending on the strain. For instance, only *Stenotrophomonas* sp. AG3 showed the ability to produce siderophores at 750 mM NaCl (SI 0.7 ± 0.1), while ACC deaminase activity and nitrogen-fixation were impaired in all strains at the highest salt concentration. EPS production was only slightly affected by salt, but in opposite directions: it started to decrease in *Stenotrophomonas* sp. AG3 at NaCl concentrations of 250 mM (from 1.2 to 0.7 g 100 mL^{-1}) and was higher and enhanced by salinity in *Exiguobacterium* sp. S60 (from 0.85 to 1.4 g 100 mL^{-1}) until 750 mM NaCl.

Regarding IAA production, salt caused a general increase in all strains except for *Exiguobacterium* sp. S56a and *Exiguobacterium* sp. S60, in which no effect was observed at 250 and 750 mM NaCl. Given that biofilm formation is an important trait that directly influences the interaction with roots, we also examined how salt affects this property in the different strains. All of them were able to form biofilms in vitro, up to 750 mM concentration of salt in the medium. However, the two strains displaying a higher capacity of biofilm formation in the absence of salt (*Stenotrophomonas* sp. AG3 and *Exiguobacterium* sp. S58) were negatively affected by salt, while the other two strains increased biofilm formation already at 250 mM NaCl. To confirm that salt does not impair biofilm formation on the surface of roots, we inoculated them with a moderate (*Stenotrophomonas* sp. AG3) and a weak biofilm producer (*Exiguobacterium* sp. S56a) and performed SEM analysis (Fig. 2). As expected, we were able to detect the adhesion of groups of bacterial cells to the root surface, both with and without salt.

These results indicate that the four bacterial strains selected for this study are able to withstand high salt concentrations and still display measurable PGP activities and biofilm formation, encouraging further tests of their use as probiotics in plant cultivation.

Effect of PGP Bacteria on Soybean Seed Germination and Plant Growth Under Salt Stress

High and uniform germination and emergence in the field are considered determinants for soybean yield, especially under salt stress conditions (Shu et al. 2017). Particularly, the seedling stage of soybean is considered to be much more sensitive to salt stress than the germination stage (Hosseini et al. 2002). It has been reported that soybean seedling growth starts to decline at 220 mM NaCl compared with the unstressed control, whereas stunted growth was observed at 300 mM NaCl (Phang et al. 2008; Kasotia et al. 2016; Chang 1994; Ashraf 1994). Therefore, to investigate the effect of the selected bacterial strains on soybean cultivation, we inoculated *Glycine max* L. seeds with the different bacterial isolates and examined their germination rates under unstressed and salt stressed conditions (250 mM).

In non-stress conditions, the germination of soybean seeds was optimal for both the inoculated and non-inoculated seeds (around 90%) and the highest germination rate was scored 3 days after sowing (Fig. 3A). We found that only *Stenotrophomonas* sp. AG3 significantly increased the percentage of germinated seeds in absence of salt. The inoculated seeds sown under NaCl displayed the highest germination percentage 5 days after sowing (Fig. 3B). However, the germination percentage of inoculated seeds was significantly increased with respect to non-inoculated seeds and eventually they reached higher germination rates. While control seeds began to germinate at day 4, the inoculated seeds began to germinate one day earlier. In the presence of salt, the seeds treated with *Exiguobacterium* sp. S56a, S58, S60 and *Stenotrophomonas* sp. AG3 significantly enhanced germination compared to untreated seeds by 42.8%, 42.8%, 35%, and 35%, respectively (Fig. 3B). Therefore, we can conclude that the bacteria were able to alleviate NaCl toxicity in terms of germination rate.

We also scored the bacterial PGP activity at later plant developmental stages under no treatment and under salt stress challenging conditions. In particular, root length and relative root surface (Fig. 4A and B) were enhanced by inoculation with *Exiguobacterium* sp. S56a, *Stenotrophomonas* sp. AG3, and *Exiguobacterium* sp. S60 both in the absence and in the presence of 250 mM NaCl. Similarly, the highest values of root dry biomass in the absence of salt were detected when the soybean roots were inoculated with

Table 1 PGP properties under control and different NaCl concentrations

	Isolate															
	Stenotrophomonas sp. AG3				*Exiguobacterium* sp. S56a				*Exiguobacterium* sp. S58				*Exiguobacterium* sp. S60			
	-NaCl	250 mM NaCl	750 mM NaCl	1500 mM NaCl	-NaCl	250 mM NaCl	750 mM NaCl	1500 mM NaCl	-NaCl	250 mM NaCl	750 mM NaCl	1500 mM NaCl	-NaCl	250 mM NaCl	750 mM NaCl	1500 mM NaCl
IAA production (µg/mL)	10.7 ± 0.4	53.6 ± 1.7	45.2 ± 6.2	nd	3.2 ± 0.9	9.4 ± 0.3	2.2 ± 0.6	nd	20.0 ± 1.5	13.1 ± 0.2	37.7 ± 1.1	nd	2.5 ± 0.2	52 ± 5.2	9.7 ± 0.1	nd
Phosphate solubilization[a]	2.5 ± 0.5	2.2 ± 0.3	1.8 ± 0.3	0.8 ± 0.1	1.5 ± 0.3	1.3 ± 0.2	1 ± 0.2	nd	1.5 ± 0.3	1.5 ± 0.2	1 ± 0.1	0.9 ± 0.1	2.3 ± 0.3	2.1 ± 0.2	2 ± 0.3	nd
Siderophores production[b]	2.2 ± 0.3	1.1 ± 0.2	0.7 ± 0.1	nd	1.5 ± 0.3	1.3 ± 0.2	nd	nd	2.0 ± 0.4	2.4 ± 0.3	nd	nd	1.2 ± 0.3	1 ± 0.2	nd	nd
Nitrogen-fixation[c]	+ + +	+ +	nd	nd	+ +	+ +	nd	nd	+	+	nd	nd	+	+	nd	nd
ACC deaminase (α-ketobutyrate nmol mg$^{/}$h)	50.1 ± 2.1	42.3 ± 3.2	nd	nd	39.2 ± 1.5	20.1 ± 2.5	nd	nd	72.5 ± 3.5	50.5 ± 5.1	nd	nd	36.3 ± 2.9	32.3 ± 2.5	nd	nd
EPS production (g/100 mL)	1.5 ± 0.1	1.2 ± 0.1	0.7 ± 0.2	nd	1.5 ± 0.2	1.7 ± 0.3	1.9 ± 0.3	nd	nd	nd	nd	nd	0.9 ± 0.1	0.85 ± 0.2	1.4 ± 0.3	nd
Biofilm production (OD at 570 nm)	0.6 ± 0.1	0.3 ± 0.04	0.2 ± 0.02	nd	0.1 ± 0.01	0.2 ± 0.03	0.3 ± 0.04	nd	0.4 ± 0.07	0.2 ± 0.03	0.2 ± 0.01	nd	0.3 ± 0.04	0.5 ± 0.08	0.5 ± 0.07	nd

The values reported in the table are the averages ± standard error of at least three independent bacterial cultures

nd no detection

[a]Phosphate solubilization index was determined by using the formula [PSI = (Colony diameter + halo zone diameter)/Colony diameter]

[b]Siderophores production index was determined by using the formula [SI = (Colony diameter + orange halo zone diameter)/Colony diameter]

[c]N_2 fixation remark: *nd* no detectable N_2 fixation, + poor N_2 fixation, ++ moderate N_2 fixation, +++ high N_2 fixation

Exiguobacterium sp. S56a, *Stenotrophomonas* sp. AG3, and *Exiguobacterium* sp. S60 (Figs. 4C, S1 and S2). The values of the root dry weight treated with *Exiguobacterium* sp. S58 did not differ much from the control roots. Under salt stress conditions, the values of root dry weight decreased, in comparison to non-salt treatment, and again the highest values corresponded to the roots inoculated with *Exiguobacterium* sp. S60 and S56a, and *Stenotrophomonas* sp. AG3: 28.8, 22.2, and 19.3 mg, respectively (Figs. 4, S1 and S2).

Effect of Bacterial Infection on Plant Gene Expression Under Non-salt and Salt Stress Growth Conditions

To better understand the plant response to the bacterial inoculations, we analyzed the expression of soybean genes involved in salt-stress response and plant development. We selected six soybean genes, *Glyma.03G226000, Glyma.02G228100*, *Glyma.03G031000*, *Glyma.04G180400*, *Glyma.08G189600,* and *Glyma.17G173200,* for gene expression analysis by RTqPCR according to previous studies in which they were found to be highly expressed in a salt-tolerant soybean line at 250 mM (Zeng et al. 2019).

The *Glyma.03G226000* gene encodes a "glycosyl hydrolase" (Zeng et al. 2019) involved in the alteration of specific b-linked polysaccharides during cell expansion in development (Leah et al. 1995). The results shown in Fig. 5A indicate that the gene was highly expressed in the roots inoculated with *Stenotrophomonas* sp. AG3, *Exiguobacterium* sp. S56a and S60 under non-stress and salt stress conditions. However, the expression of this gene was not significantly different in the roots inoculated with *Exiguobacterium* sp. S58 in both conditions.

The remaining five genes evaluated are involved in the salt stress response. We observed that *Glyma.02G228100* encoding a glutamine-dependent asparagine synthase 1 (ASN1) was downregulated by all inoculants under non-salt conditions in comparison with the non-inoculated control (Fig. 5B). However, in response to salt stress, inoculation with three out of our four strains (*Stenotrophomonas* sp. AG3 and *Exiguobacterium* sp. S56a and S60) increased asparagine synthase expression, as a marker of salt stress tolerance. By contrast, in *Exiguobacterium* sp. S58, *Glyma.02G228100* expression was non-significantly different.

The gene *Glyma.03G031000* encodes a cytochrome P450 (CYP450) and actively participates in the biosynthesis of plant growth factors, such as the brassinosteroids (BRs) (Takahashi et al. 2005). BRs have a role in stress resistance, including salinity, extreme temperatures, and pathogen attack (Clause and Sasse, 1998). However, our results showed a similar expression level between the non-salt and salt treatments (Fig. 5C). The addition of the bacterial inoculants did not affect the expression of this gene substantially under non-salt or salt conditions.

Another stress-reporter gene is *Glyma.04G180400,* encoding a BURP domain-containing protein. The soybean genome contains 23 members of the BURP domain-containing gene family, 17 of them responsive to stress (Xu et al. 2010). As shown in Fig. 5D, expression of *Glyma.04G180400* did not change under non-salt conditions in the presence of the bacteria. However, the gene was highly induced by salt in roots with *Stenotrophomonas* sp. AG3, *Exiguobacterium* sp. S56a and S60. As with other genes, the roots inoculated with *Exiguobacterium* sp. S58 did not present significant gene expression alterations under salt stress conditions.

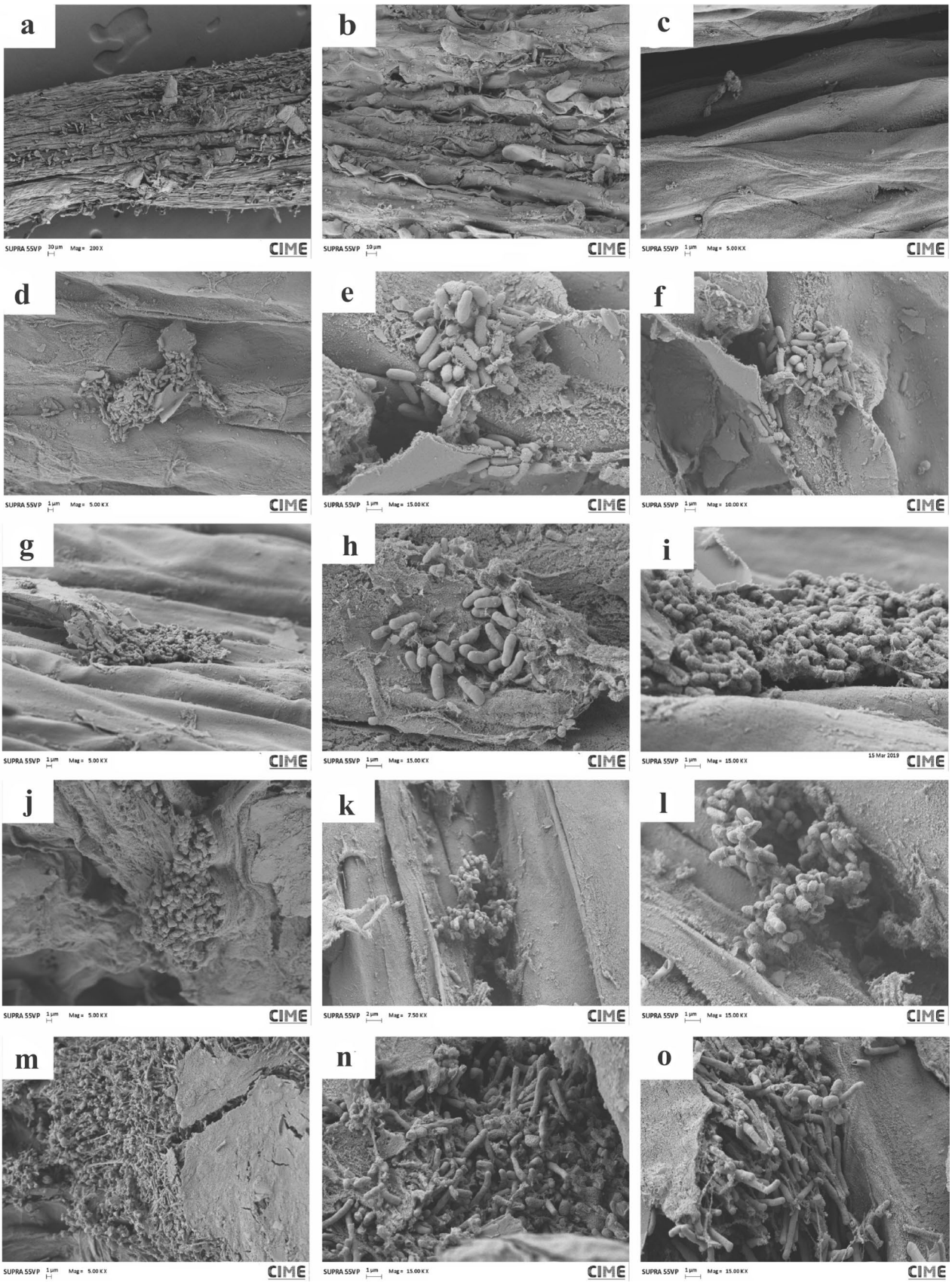
Glyma.08G189600 gene encodes a lipoxygenase involved in the generation of signaling molecules that act in the responses of plants to different situations of biotic and abiotic stress (Lim et al. 2015). Similar to the previous gene, no change in gene expression was found under non-stress conditions, but it was highly induced by salt in roots inoculated with *Stenotrophomonas* sp. AG3 and *Exiguobacterium* sp. S56a and S60 (Fig. 5E).

Glyma.17G173200, encoding a dihydroflavonol 4-reductase, plays a key role in the formation of common and condensed anthocyanins (Holton et al. 1995). Anthocyanins function as antioxidants in vegetative tissues to scavenge reactive oxygen species (ROS) and as antimicrobial agents during defense responses (Tian et al. 2017). Our data for *Glyma.17G173200* gene expression (Fig. 5F) showed non-significant changes in expression levels under non-salt and salt treatments for *Exiguobacterium* sp. S60 and S58. By contrast, the gene was highly induced by salt treatments in *Exiguobacterium* sp. S56a and *Stenotrophomonas* sp. AG3 infected roots.

These results, combined with the growth parameters evaluated, indicate that the presence of *Stenotrophomonas* sp. AG3 and *Exiguobacterium* sp. S60 and S56a can promote growth in soybean roots in the presence of inhibitory salt concentrations by pre-induction of stress-responsive genes.

Discussion

The salinization of soils is one of the most severe stress factors increasingly threatening crop productivity and future food safety. Although salt-tolerant crops have been planted in different circumstances (Fita et al. 2015), this is not always an option due to limitations in the tolerance range and in the availability of salt-tolerant varieties in the species of interest. Therefore, the use of PGP microorganisms still represents an attractive alternative. Bacteria isolated from extreme environments from different genera, such as *Rhizobium, Bacillus, Pseudomonas, Burkholderia,*


a
SUPRA 55VP 30 µm Mag = 200 X CIME
b
SUPRA 55VP 10 µm CIME
c
SUPRA 55VP 1 µm Mag = 5.00 KX CIME
d
SUPRA 55VP 1 µm Mag = 5.00 KX CIME
e
SUPRA 55VP 1 µm Mag = 15.00 KX CIME
f
SUPRA 55VP 1 µm Mag = 10.00 KX CIME
g
SUPRA 55VP 1 µm Mag = 5.00 KX CIME
h
SUPRA 55VP 1 µm Mag = 15.00 KX CIME
i
SUPRA 55VP 1 µm Mag = 15.00 KX 15 Mar 2019 CIME
j
SUPRA 55VP 1 µm Mag = 5.00 KX CIME
k
SUPRA 55VP 2 µm Mag = 7.50 KX CIME
l
SUPRA 55VP 1 µm Mag = 15.00 KX CIME
m
SUPRA 55VP 1 µm Mag = 5.00 KX CIME
n
SUPRA 55VP 1 µm Mag = 15.00 KX CIME
o
SUPRA 55VP 1 µm Mag = 15.00 KX CIME

◂**Fig. 2** Biofilm Imaging with SEM**.** Control roots (**a–c**). *Stenotrophomonas* sp. AG3 **(d–f)** and *Exiguobacterium* sp. S56a (**j–l**) cell adhesion on root surface grown in non-salt conditions. *Stenotrophomonas* sp. AG3 (**g–i**) and *Exiguobacterium* sp. S56a (**m–o**) cell adhesion on root surface grown under 250 mM of NaCl

Achromobacter, Methylobacterium, and *Variovorax* are able to improve crop performance under stress (Shrivastava et al. 2015). However, the search for new species with PGP potential is justified by the possibility that newly found endemic species are better adapted to local cultivation conditions and soils. The present study reports the effectiveness of halophilic bacteria from Andean soil to ameliorate the stress caused by salt in soybean germination and growth.

An important issue that has not been completely solved yet is the determination of the mechanisms by which these halophilic bacteria ameliorate salt stress. It has been proposed that PGP microorganisms can improve the performance of crop plants under stress through different mechanisms: the production of phytohormones, the elimination of ethylene via ACC deaminase, the improvement of nutrient assimilation, and the stimulation of the plant's defense mechanisms, among others (Egamberdieva et al. 2019). Our results suggest that some of these mechanisms might explain the beneficial effect of halophilic bacteria.

Firstly, one possibility is that the PGP activity of these bacteria does not strictly occur under salt stress. Rather, the improved performance of plants growing in the presence of the strains tested here would be the combination of two factors: the intrinsic growth promoting capacity of these bacteria, and their own ability to survive and maintain at higher saline concentrations a productive physiological interaction with the plants. Unlike other bacteria, whose growth is largely affected by salt (Steinborn and Roughley 1974), the observation that all the species analyzed here can still form biofilms on soybean roots at higher salt concentrations points in this direction. Once the bacteria colonize the roots, the improvement of growth-related traits can be attributed to bacterially produced IAA (Keswani et al. 2020). The stimulation of shoot and root growth in wheat by IAA produced by *Pseudomonas* strains has also been reported in saline soil (Egamberdieva 2009). We cannot rule out at this point that these halophilic strains also produce additional phytohormones that may add to the observed effects.

Secondly, it has been reported that at high salt concentrations, ethylene is synthesized by the plant, and it can trigger different processes that may inhibit growth and plant survival (Glick 2012). Some PGPB can produce the enzyme ACC deaminase, which catalyzes the conversion of ACC, an immediate precursor of the synthesis of ethylene in plants, into α-ketobutyrate and ammonia (Penrose and Glick 2003). Our results show that the extremophilic microorganisms studied here could alleviate saline stress partly through the production of ACC deaminase, preventing ethylene-dependent growth inhibition. Previous reports have attributed to the combined action of IAA and ACC deaminase the promotion of sunflower growth by *Serratia* strains (Mendoza-Hernández et al. 2016).

A third mechanism used by salt-tolerant PGP bacteria involves the increase in availability of nutrients such as phosphate, nitrogen, or ions (Szymańska et al. 2013). The ability of the tested bacterial strains to solubilize phosphate and produce siderophores under high salt concentrations is a possible consequence of the greater adaptability to stress conditions of these bacteria because they were isolated from extreme environments (Andean Peatland). Particularly, *Stenotrophomonas* sp. AG3 and *Exiguobacterium* sp. S58 were able to solubilize phosphate as the salt concentration was increased (up to 1500 mM). This ability was also observed in bacteria isolated from salt-affected and contaminated soils. *Alteromonas* sp. PSBCRG$_2$-1, *Serratia marcescens,* and *Pseudomonas aeruginosa* continued solubilizing phosphate up to 1000 and 1300 mM NaCl (Nakbanpote et al. 2014; Srinivasan et al. 2012).

Finally, our examination of a series of salt-stress marker genes in the presence of halophilic strains indicates differential effects, depending on the strain. *Glyma.03G226000*, which encodes a "glycosyl hydrolase", behaved as an indicator of plant growth promotion, based on the good correlation between its induction and the growth parameters evaluated. Roots inoculated with *Stenotrophomonas* sp. AG3 and *Exiguobacterium* sp. S56a and S60 overexpressed the *Glyma.03G226000* gene in the absence and in the presence of salt. Besides, these strains ameliorated the salt-associated defects in root length, relative root surface area, and the dry biomass of soybean in comparison with control roots. An opposite effect was observed in the roots inoculated with *Exiguobacterium* sp. S58 under two treatments. On the other hand, the induction of *Glyma.02G228100*, encoding a glutamine-dependent asparagine synthase, could well be related to the increased plant salt tolerance. Under mineral deficiency, salt stress, or drought, the concentration of asparagine increases in plant tissues because asparagine detoxifies the ammonia produced when abiotic stresses lead to ammonia accumulation in plants (Wang et al. 2005). The up-regulation of asparagine synthase genes by salinity and osmotic stresses has also been reported in wheat seedlings (Wang et al. 2005). Besides, the presence of the strains may also increase the salt tolerance by overexpressing BURP proteins (*Glyma.04G180400*), which help in the adaptation and development of stressed plants (Wang et al. 2015), and enzymes (*Glyma.17G173200*) involved in the formation of antioxidant compounds (Tian et al. 2017). In addition, the upregulation of a lipoxygenase gene (*Glyma. 08G189600*) involved in the generation of oxylipins, which act as signaling molecules in the responses of plants to stress, could

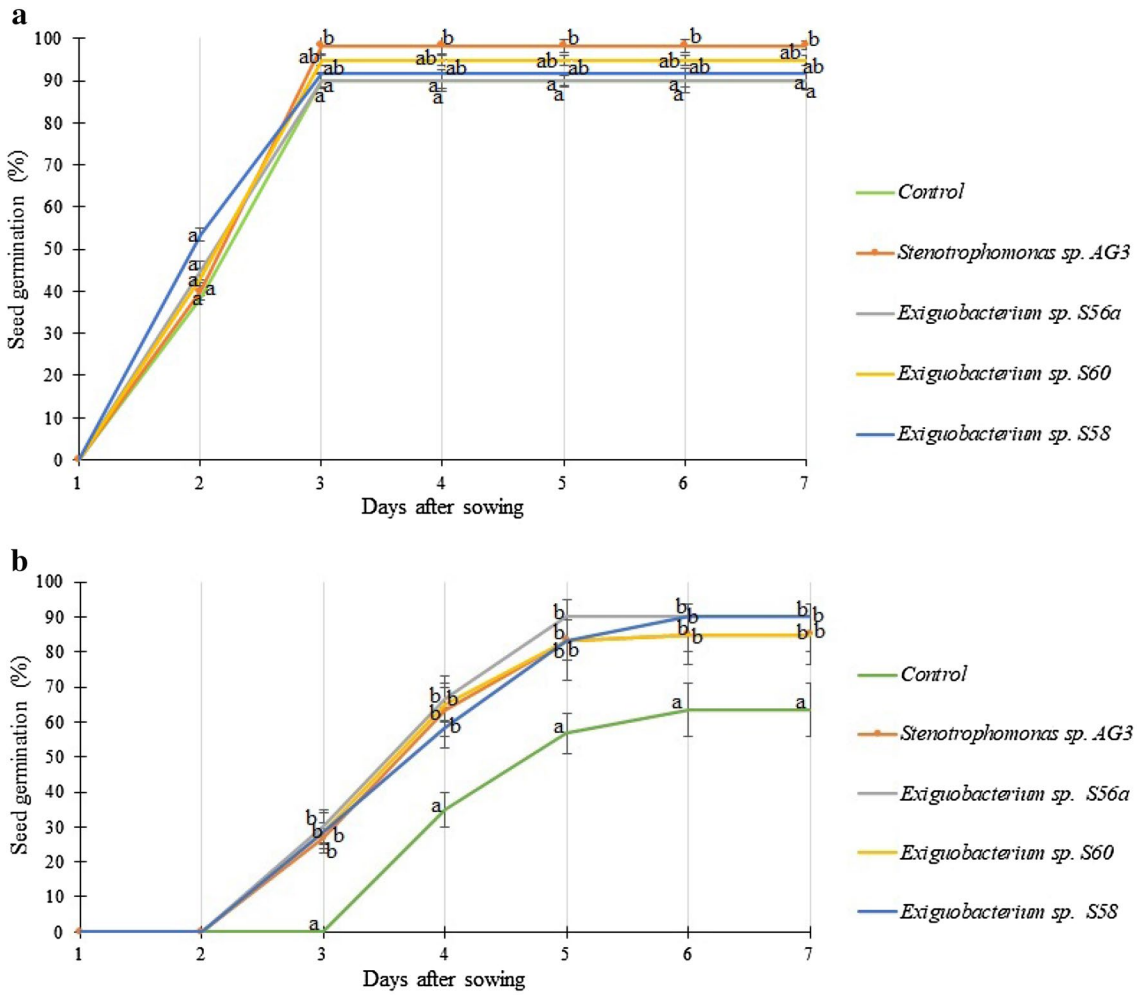


Fig. 3 Germination assay seeds (Glycine Max L.) under **a** non-salt and **b** salt (250 mM) stress conditions. The percentage of germinated seeds was scored at the indicated time points. Data represent the mean ± standard error of three independent biological replicates with at least 20 seeds. Means with same letters did not differ significantly ($p < 0.05$) according to Tukey's HSD test

indicate that the presence of microorganisms helps to enhance the plant immune system.

Conclusion

These results show that *Stenotrophomonas* sp. AG3 and *Exiguobacterium* sp. S56a and S60 have the potential to improve the growth of soybean in the absence and in the presence of NaCl stress These bacteria are able to ameliorate the salinity stress and promote growth probably through the synthesis of plant hormones (IAA), induction of ACC deaminase activity, phosphate solubilization, and the production of siderophores, EPS, and biofilms. Moreover, it is likely that extremophilic bacteria can also mitigate the salt toxicity by inducing the plant's stress molecular responses as indicated by the expression of salt-stress soybean marker genes (*Glyma.02G228100, Glyma.04G180400* and *Glyma.08G189600*). This work expands the understanding of the growth-promoting properties and salt tolerance mechanism of extremophiles isolated from Andean soil and provides further knowledge for the use of halophilic bacteria as bioinoculants for sustainable crop production under saline environments.

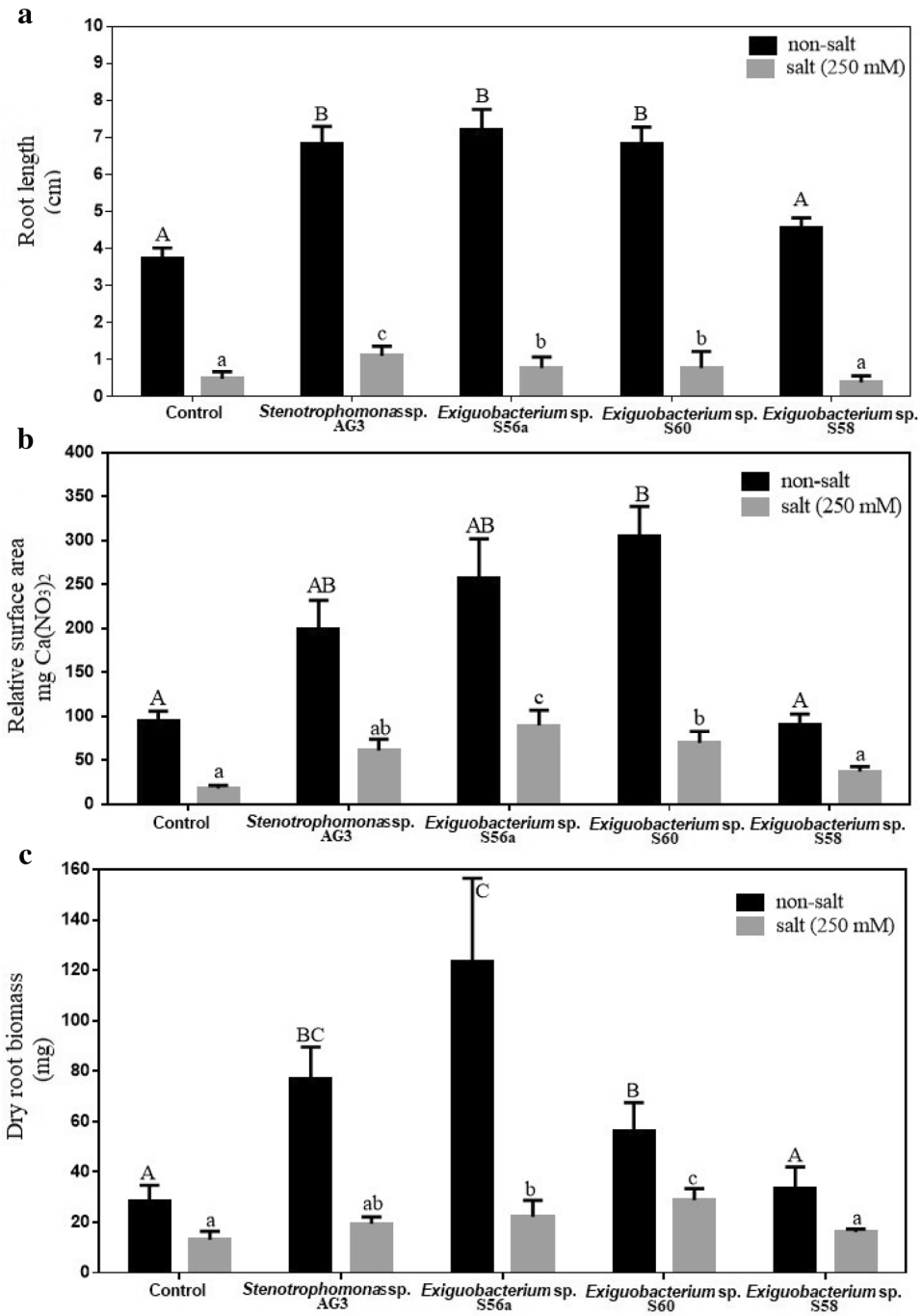


Fig. 4 Plant growth promoting effects in **a** root length, **b** relative root surface area and **c** dry biomass of soybean under non-salt and salt treatment (250 mM of NaCl). Data represent mean ± standard error ($n = 3$). Values with same letters did not differ significantly according to Tukey's HSD test with $p < 0.05$. Lowercase letters on the bar compare each bacterial strain for salinity treatment. Uppercase letters compare each bacterial strain for non-salinity treatment

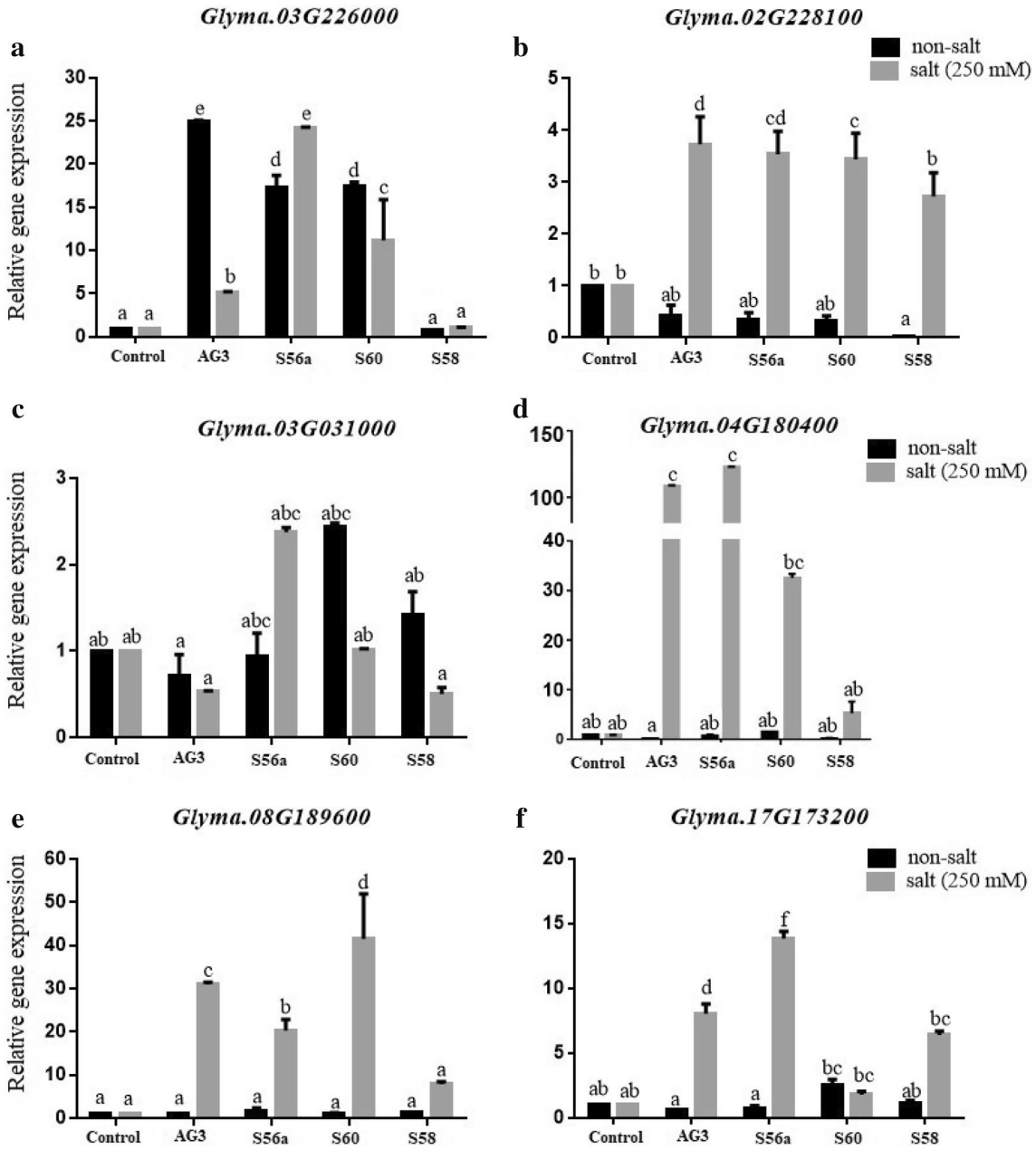


Fig. 5 Relative gene expression in soybean roots analyzed by RTqPCR normalized to housekeeping gene and to control conditions in response to non-salt and salt treatment (250 mM of NaCl). Data represents mean ± standard error ($n = 3$). Values with same letters did not differ significantly according to Tukey's HSD test with $p < 0.05$ compared to control roots

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-022-10605-5.

Acknowledgements The authors acknowledge the generous financial support by the PICT V Bicentenario 2010 1788 Project (FONCyT, Argentina). This work was performed in the context of the project called "Análisis de Adaptación al Cambio Climático en Humedales Andinos". ID: 6188775-8-LP13. Ministerio del Medio Ambiente, Región de Antofagasta (www.mma.gob.cl/).

Author Contributions All the authors contributed to the design and implementation of the research, the analysis of the results, and the writing of the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Ashraf M (1994) Genetic variation for salinity tolerance in spring wheat. Hereditas 120:99–104

Belfiore C, Fernandez A, Santos AP, Contreras M, Farías ME (2018a) Characterization and comparison of microbial soil diversity in two andean peatlands in different states of conservation-vega tocorpuri. J Environ Prot 6:194–210

Belfiore C, Santos AP, Contreras M, Farias ME (2018b) Isolation and characterization of plant growth promoting bacteria isolated from andean soil as potential inoculants of soybean seeds. Environ Nat Resour J. https://doi.org/10.5539/enrr.v8n3p203

Carley HE, Watson TW (1966) A new gravimetric method forestimating root-surface areas. Soil Sci 102:289–291

Chang RZ (1994) Effect of salt on agricultural characters and chemical quality of seed in soybean. Soybean Sci 13:101–105

Clause SD, Sasse JM (1998) Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Biol 49:427–251

Del-amor FM, Cuadra-Crespo P (2012) Plant growth-promoting bacteria as a tool to improve salinity tolerance in sweet pepper. Funct Plant Biol 39:82–90

Dobereiner J (1995) Isolation and identification of aerobic nitrogen fixing bacteria from soil and plant. In: Alef K, Nannipieri P (eds) Methods in applied soil microbiology and biochemistry. Academic Press, Cambridge, pp 134–141

Edbeib MF, Wahab RA, Huyop F (2016) Halophiles: biology, adaptation, and their role in decontamination of hypersaline environments. World J Microbiol Biotechnol 32:1–23

Egamberdieva D (2007) The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl Soil 36:184–189

Egamberdieva D (2009) Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol Plant 31:861–864

Egamberdieva D, Kucharova Z (2009) Selection for root colonizing bacteria stimulating wheat growth in saline soils. Biol Fertil Soil 45:563–571

Egamberdieva D, Wirth S, Bellingrath-Kimura SD, Mishra J, Arora NK (2019) Salt- tolerant plant growth promoting rhizobacteria for enhancing crop productivity of saline soils. Front Microbiol 10:2791

Fita A, Rodríguez-Burruezo A, Boscaiu M, Prohens J, Vicente O (2015) Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. Front Plant Sci 6:978

Garcia J, Schmidt JE, Gidekel M, Gaudin AC (2021) Impact of an antarctic rhizobacterium on root traits and productivity of soybean (*Glycine max* L.). J Plant Nutr 44:1818–1825

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica. https://doi.org/10.6064/2012/963401

Han HS, Lee KD (2005) Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Res J Agr Biol Sci 1:210–215

Harman GE, Björkman T (1998) Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement. Trichoderma Gliocladium 2:229–265

Holton TA, Cornish EC (1995) Genetics and biochemistry of anthocyanin biosynthesis. Plant Cell 7:1071–1083

Hossain MS (2019) Present scenario of global salt affected soils, its management and importance of salinity research. Int Res J Biol Sci 1:1–3

Hosseini MK, Powell AA, Bingham IJ (2002) Comparison of the seed germination and early seedling growth of soybean in saline conditions. Seed Sci Res 12(3):165–172

Ilangumaran G, Smith DL (2017) Plant growth promoting rhizobacteria in amelioration of salinity stress: a systems biology perspective. Front Plant Sci 8:1–14

Kasotia A, Varma A, Tuteja N, Choudhary DK (2016) Amelioration of soybean plant from saline-induced condition by exopolysaccharide producing *Pseudomonas*-mediated expression of high affinity K+-transporter (HKT1) gene. Curr Sci 111:1961–1967

Keswani C, Singh SP, Cueto L, García-Estrada C, Mezaache-Aichour S, Glare TR, Borriss R, Singh SP, Blázquez MA, Sansinenea E (2020) Auxins of microbial origin and their use in agriculture. Appl Microbiol Biotechnol 104:1–17

Khan MA, Sahile AA, Jan R, Asaf S, Hamayun M, Imran M, Adhikari A, Kang SM, Kim MK, Lee IJ (2021) Halotolerant bacteria mitigate the effects of salinity stress on soybean growth by regulating secondary metabolites and molecular responses. BMC Plant Biol 21:1–15

Kumari S, Vaishnav A, Jain S, Varma A, Choudhary DK (2015) Bacterial- mediated induction of systemic tolerance to salinity with expression of stress alleviating enzymes in soybean (*Glycine max* L. Merrill). J Plant Growth Regul 34:558–573

Leah R, Kigel J, Svendsen I, Mundy J (1995) Biochemical and molecular characterization of a barley seed B-glucosidase. J Biol Chem 270:15789–15797

Lim CH, Han S, Hwang IS, Kim DS, Lee HBK, SH, (2015) The pepper lipoxygenase CaLOX1 plays a role in osmotic, drought and high salinity stress response. Plant Cell Physiol 56:930–942

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using realtime quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408

Lugtenberg BJJ, Dekkers L, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomanas*. Annu Rev Phytopathol 39:461–490

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

Mendoza-Hernandez CP, Perea-Velez YS, Arriola-Morales J, Martínez-Simon S, Perez- Osorio G (2016) Assessing the effects of heavy metals in ACC deaminase and IAA production on plant growth-promoting bacteria. Microbiol Res 188–189:53–61

Nakbanpote W, Panitlurtumpai N, Sangdee A, Sakulpone N, Sirisom P, Pimthong A (2014) Salt-tolerant and plant growth-promoting bacteria isolated from Zn/Cd contaminated soil: identification and effect on rice under saline conditions. J Plant Interact 9:379–387

Nautiyal CS, Srivastava S, Chauhan PS, Seem K, Mishra A, Sopor SK (2013) Plant growth- promoting bacteria *Bacillus*

amyloliquefaciens NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. Plant Physiol Biochem 66:1–9

O'Toole GA (2011) Microtiter dish biofilm formation assay. J Visual Exp Jove 47:24–37

Papiernik SK, Grieve CM, Lesch SM, Yates SR (2005) Effects of salinity, imazethapyr, and chlorimuron application on soybean growth and yield. Commun Soil Sci Plant Anal 36:951–967

Penrose DM, Glick R (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol Plant 118:10–15

Phang TH, Shao G, Lam HM (2008) Salt tolerance in soybean. J Integr Plant Biol 50(10):1196–1212

Puchulú ME (2008) Salinización de los suelos. Su incidencia en la provincia de Tucumán y en la República Argentina. Acta Geológica Lilloana 21:81–94

Rangarajan S, Saleena LM, Vasudevan P, Nair S (2003) Biological suppression of rice diseases by *Pseudomonas* spp. under saline soil conditions. Plant Soil 251:73–82

Rehman S, Harris PJC, Bourne WF, Wilkin J (1996) The effect of sodium chloride on germination and the potassium and calcium contents of acacia seeds. Seed Sci Technol 25:45–57

Rodríguez DM, Schulz GA, Tenti Vuegen LM (2019) Distribución de suelos afectados por sales en Argentina. Actas De La Red Argentina De Salinidad (RAS) 6:24–30

Sapsirisopa S, Chookietwattana K, Maneewan K, Khangkhan P (2009) Effect of salt- tolerant *Bacillus* inoculum on rice KDML 105 cultivated in saline soil. Asian J Food Agric Ind Organ 2:69-S74

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160(1):47–56

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

Shu K, Qi Y, Chen F, Meng Y, Luo X, Shuai H, Zhou W, Ding Jun DuJ, Liu J, Yang F, Wang Q, Ding J, Du J, Liu J, Yang F, Yang F, Wang Q, Liu W, Yong T, Wang X, Feng Y, Yang W (2017) Salt stress represses soybean seed germination by negatively regulating GA biosynthesis while positively mediating ABA biosynthesis. Front Plant Sci 8:1372

Siddikee MA, Glick BR, Chauhan PS, Yim WJ, Sa T (2011) Enhancement of growth and salt tolerance of red peper seedlings (*Capsicum annuu* L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1-aminocyclopropane-1-carboxylic acid deaminase activity. Plant Physiol Biochem 49:427–434

Srinivasan R, Yandigeri MS, Kashyap A (2012) Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. Saudi J Biol Sci 19(4):427–434

Steinborn J, Roughley RJ (1974) Sodium chloride as a cause of low numbers of rhizobium in legume inoculants. J Appl Microbiol 37:93–99

Szymańska S, Piernik A, Hrynkiewicz K (2013) Metabolic potential of microorganisms associated with the halophyte *Aster tripolium* L. in saline soils. Ecol Quest 18(1):9–19

Takahashi N, Nakazawa M, Shibata K, Yokota T, Ishikawa A, Suzuki K, Kawashima M, Ichikawa T, Shimada H, Matsui M (2005) shk1-D, a dwarf *Arabidopsis* mutant caused by activation of the CYP72C1 gene, has altered brassinosteroid levels. Plant J 42:13–22

Tang YW, Bonner J (1948) The enzymatic inactivation of indole acetic acid. II. The physiology of the enzyme. Am J Bot 35:570–578

Tapia-Vázquez I, Sánchez-Cruz R, Arroyo-Domínguez M, Lira-Ruan V, Sánchez- Reyes A, del Rayo S-CM, Padilla-Chacón D, Batista-García RA, Folch- Mallol JL (2020) Isolation and characterization of psychrophilic and psychrotolerant plant-growth promoting microorganisms from a high-altitude volcano crater in Mexico. Microbiol Res 232:126394

Tian J, Chen M, Zhang J, Li K, Song T, Zhang X, Yao Y (2017) Characteristics of dihydroflavonol 4-reductase gene promoters from different leaf colored *Malus* crabapple cultivars. Hortic Res. 4:17070

Ungar IA (1996) Effect of salinity on seed germination, growth, and ion accumulation of *Atriplex patula* (Chenopodiaceae). Am J Bot 83:604–607

Vázquez P, Holguin G, Puente E, Lopez-Cortez A, Bashan Y (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in semiarid costal lagoon. Biol Fertil Soils 30:460–468

Verhoef R, De Waard P, Schols HA, Siika-aho M, Voragen AG (2003) *Methylobacterium* sp. isolated from a Finnish paper machine produces highly pyruvated galactan exopolysaccharide. Carbohydr Res 338:1851–1859

Wang H, Liu D, Sun J, Zhang A (2005) Asparagine synthetase gene TaASN1 from wheat is up regulated by salt stress, osmotic stress, and ABA. J Plant Physiol 162(1):81–89

Wang L, Wu N, Zhu Y, Song W, Zhao W, Li Y, Hu Y (2015) The divergence and positive selection of the plant-specific BURP-containing protein family. Ecol Evol 5(22):5394–5412

Xu HL, Li YX, Yan YM, Wang K, Gao Y, Hu Y (2010) Genome-scale identification of Soybean BURP domain-containing genes and their expression under stress treatments. BMC Plant Biol 10:197–212

Yadav AN (2017) Beneficial role of extremophilic microbes for plant health and soil fertility. J Agric Sci 1(1):30

Zeng A, Chen P, Korth KL, Ping J, Thomas J, Wu C, Srivastava S, Pereira A, Hancock F, Brye K, Ma J (2019) RNA sequencing analysis of salt tolerance in soybean (*Glycine max*). Genomics 111(4):629–635