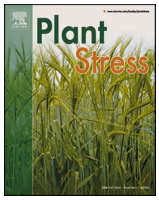
Plant Stress 21 (2026) 101371

Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress



Exploring quinoa's plant growth-promoting bacteria: a *Pseudomonas* seed endophyte promotes salinity tolerance and modifies root architecture in *Arabidopsis thaliana*

Karina B. Ruiz [a,1,*], Giorgia Novello [b,1], Ricardo Tejos [a], Sebastian Segovia-Ulloa [a], Sebastián Sepúlveda-Villegas [a], Matías Araya-Araya [a], Gabriela Aguirre-Martínez [a], Fabiana Antognoni [c], Stefania Biondi [d], Patrizia Cesaro [b], Elisa Bona [e,*], Guido Lingua [b], Elisa Gamalero [b]

[a] *Química y Farmacia, Facultad de Ciencias de la Salud, Universidad Arturo Prat, Av. Arturo Prat Ch. 2120, Iquique 1100000, Chile*
[b] *Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Viale T. Michel 11, 15121 Alessandria, Italy*
[c] *Scienze per la Qualità della Vita, Università di Bologna, Rimini Campus, Corso d' Augusto 237, 47921 Rimini, Italy*
[d] *Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Via Irnerio 42, 40126 Bologna, Italy*
[e] *Dipartimento per lo Sviluppo Sostenibile e la Transizione Ecologica, Università del Piemonte Orientale, Piazza San Eusebio 5, 13100 Vercelli, Italy*

ARTICLE INFO

Keywords:
Chenopodium quinoa
Gene expression
Halotolerant bacteria
PGPB
Pseudomonas putida
Salt stress
Seed endophyte

ABSTRACT

Quinoa (*Chenopodium quinoa* Willd) is a halophytic crop species native to the Andes. Its salt tolerance mechanisms have been widely studied but less is known about the beneficial microorganisms that potentially contribute to its resilience. Seed endophytes are the least investigated plant-associated microbes, despite their importance in supporting germination, seedling establishment, and in priming the plant for stress tolerance. In this work fifteen strains of culturable endophytic bacteria were isolated from surface-sterilised quinoa seeds and identified by MALDI-TOF. All strains were halotolerant and exhibited one or more plant growth-promoting (PGP) activity, *viz.* phosphate and potassium solubilization, indole-acetic acid, organic acid, exopolysaccharide, and siderophore production, ACC deaminase activity, and nitrogen fixation. A *Pseudomonas* strain (code A7) with the most PGP features was identified by 16S rDNA as *Pseudomonas putida*. To investigate the stress-mitigating potential of isolate A7, the model plant *Arabidopsis* was grown with 0 or 100 mM NaCl. After 12 days under salt stress, A7 restored fresh biomass and chlorophyll content to control levels. Inoculation with A7 enhanced the number of lateral roots on saline and non-saline medium. Transcript levels of genes belonging to several functional categories, analysed by quantitative real-time-PCR, were affected by salt or by inoculation with A7 alone; in several cases (*AtCYCD2, AtANR1, AtNTR2.1, AtHEMA, AtGRX3* and *4, AtSOS1*), salt-induced downregulation was reverted to control levels (or more) by A7.

1. Introduction

It is well-established that plants are colonized by beneficial microorganisms and that these play essential roles in their growth and development as well as resilience to biotic and abiotic stressors, including salinity (Gupta and Pandey, 2019; Khan et al., 2021; Taheri et al., 2025). There are numerous studies on such microbes, especially those that live in and around the root system, i.e., nitrogen (N)-fixing bacteria, plant growth-promoting bacteria (PGPB) including the rhizospheric plant-growth promoting rhizobacteria (PGPR) (Dimkpa et al., 2009; Khoso et al., 2024), and mycorrhizal fungi (Miño et al., 2025). The growth-promoting and stress-mitigating functions of these microorganisms derive from a number of activities that they are capable of, amongst which potassium (K) and phosphate (P) solubilization, phytohormone (e.g., auxin) production, inhibition of stress hormone (ethylene) biosynthesis by producing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, and secretion of organic acids and bioactive secondary metabolites. Under saline conditions, exopolysaccharides

* Corresponding authors.
E-mail addresses: karuiz@unap.cl (K.B. Ruiz), elisa.bona@uniupo.it (E. Bona).
[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.stress.2026.101371
Received 6 December 2025; Received in revised form 18 March 2026; Accepted 6 April 2026
Available online 7 April 2026

(EPS) secreted by PGPR can bind Na^+ and limit its uptake into the plant (Ashraf et al., 2004). They can also contrast the negative impact of salinity by enhancing protective mechanisms, such as proline accumulation and antioxidant enzyme activities (Gupta et al., 2022).

In conjunction with their physiological attributes (e.g. auxin and ACC deaminase production) making them capable of modifying root system development and reducing stress-related responses, PGPB possessing N-fixing and P-solubilizing abilities have the most significant impact on crop production, mainly by improving nutrient acquisition. Indeed, using beneficial microorganisms, either single strains or in a consortium, as biofertilizers to boost plant growth, productivity, and stress tolerance is regarded as a cost-effective, and more sustainable approach compared to chemical fertilizers (Ali et al., 2022).

Plant-growth promoting bacteria colonizing plant tissues internally are known as endophytes. These beneficial microbes have been attracting increasing attention in recent years (Fagorzi et al., 2022), in particular those associated with stress-tolerant plant species, such as quinoa (González-Teuber et al., 2018). In addition to providing new insight into their contribution to the stress resilience of such species, they may also have significant applications in agriculture, especially under stressful conditions (Maestro-Gaitán et al., 2025 and references therein). For example, Yang et al. (2016) examined the effect of six endophytic and rhizospheric plant growth-promoting bacterial strains on salt stress responses in quinoa. The two most halotolerant strains (*Enterobacter* sp. and *Bacillus* sp.) mitigated the negative effects of salinity on growth, physiological parameters, and yield by improving plant water relations and decreasing Na^+ uptake.

Seed-borne endophytes originate from the mother plant and are transmitted both horizontally and vertically. They are believed to benefit germination and seedling establishment and are likely to activate biotic and abiotic stress tolerance/resistance mechanisms early in the host plant's life cycle. Consequently, seedlings/plants are naturally primed to cope with stressful conditions, including those to which the bacteria themselves are resistant (e.g. halotolerant PGPB) (Truyens et al., 2015). Seed-borne bacteria with growth-promoting effects were reported in soybean (Kim et al., 2023), rice (Hardoim et al., 2012; Hernández et al., 2023), cactus (Puente et al., 2009), and tomato (Xu et al., 2014), while in *Nicotiana tabacum*, the phytotoxic effects of cadmium (Cd) were mitigated by seed endophytes (Mastretta et al., 2009). In a recent review, Romão et al., (2025) stated that the "seed-associated microbiota represents a critical yet underexplored frontier in plant–microbe interaction." Indeed, although studies are underway at multiple levels (Kumar et al., 2024), significant gaps remain in understanding the ecological and functional roles of seed-borne microbes whose potential importance has been less explored than that between plants and soil microorganisms.

Based on the assumption that PGPB play a significant role in the abiotic stress resilience of their hosts, an interesting line of research is the one that attempts to isolate and characterize the microbiota of naturally stress-tolerant plant species, such as those growing in deserts or arid/saline zones (Soussi et al., 2016; Eida et al., 2018). They can be isolated to test their efficacy in enhancing the tolerance of stress-sensitive plants (Qin et al., 2016; Alsharif et al., 2020; Schmitz et al., 2022). Recently, Zahra et al (2024) reviewed the potential of halotolerant PGPB as biofertilizers to improve crop productivity under salt stress.

Quinoa (*Chenopodium quinoa* Willd.) is native to the Andean region of South America where it grows from sea level to the *altiplano* (*ca.* 4,000 m a.s.l.). It is extremely tolerant to various abiotic stress factors, namely high salinity, frost, and drought (Jacobsen et al., 2003) and is, therefore, an excellent candidate for investigating the plant-associated beneficial microbes. Pitzschke (2016) discovered that quinoa seeds of the cultivar 'Real,' harvested in Bolivia, were colonised by various members of the genus *Bacillus* and that the bacteria were mobile and able to colonize all seedling organs. Recently, Maestro-Gaitán et al. (2023, 2025) explored the endophytic communities in seeds and roots from two distinct genotypes of quinoa under well-watered and drought conditions. Bacterial community composition in the rhizosphere was influenced by drought and genotype, whereas the core endophytic bacteria in seeds were conserved under varying water conditions and across genotypes.

The underlying mechanisms for the beneficial effects of bacterial endophytes are still largely elusive. Except for the study by Pitzschke (2016), who showed that, in quinoa, seed endophytes could modify the plant's redox status thanks to their catalase activity and high superoxide content, their plant-growth promoting (PGP) activities have not yet been investigated. We aimed to fill, at least in part, this knowledge gap by identifying and then assessing the PGP features of quinoa's culturable seed-borne bacterial endophytes. We then investigated the morphological, biochemical, and transcriptional effects of a selected bacterial strain exhibiting multiple PGP traits on the salt-sensitive model plant *Arabidopsis thaliana* under saline and non-saline conditions.

2. Materials and methods

2.1. Material

Seeds of *Chenopodium quinoa* var. Pandela (red variety) were collected in a cultivated field from Colchane in the Andean altiplano of Chile at the following location: latitude 19°16'34" S; longitude 68°38'16" W; 3,702 m a.s.l.

2.2. Isolation of seed endophytic bacteria

After three consecutive washes to remove all external particles (sand, insect parts, plant debris, etc.) from the samples, 2 g of whole seeds were sterilized by exposure to chlorine gas for 4 h and then placed under a laminar flow bench for 4 h. They were then washed three times with phosphate-buffered saline (PBS) solution with gentle agitation. A control experiment was performed in which surface-sterilized seeds and non-sterilized controls were directly plated onto Luria Bertani (LB) solid growth medium and incubated at 28°C for 72 h. Then, approximately 20 mg of seeds were placed in sterile 1.5-mL Eppendorf tubes to which 300 µL of 10 mM $MgCl_2$ were added. Using a sterile pestle, the seeds were ground inside the tube, until a homogeneous paste was obtained. The paste was transferred into a Falcon tube with 25 mL of 10 mM $MgCl_2$ and incubated for 15 min on a shaker. The suspension was centrifuged at 4,000 rpm for 15 min and then a 100-µL aliquot of the supernatant was placed in Eppendorf tubes with 900 µL of 10 mM $MgCl_2$ (one-fold dilution). Three further dilutions (2-fold and 4-fold) were also made. LB solid growth medium was prepared and poured into Petri dishes. Fifty µL of the 2- and 4-fold dilutions of the supernatant were used to inoculate the agar plates (a total of 40 plates, four plates for each dilution), which were then left upside down at 28°C for 7 days in an incubator (Labtech, model LIB-030M-L). Fifty µL of these dilutions were also plated on Tryptic Soy Broth (TSB) culture medium. The plates were then incubated at 28°C for 24 h and immediately used for isolation.

2.3. Preparation of bacterial stocks in solid and liquid culture

Single colonies growing on solid LB and TSB medium were isolated on fresh medium and incubated for 4 d at 28°C. Then, pure colonies were inoculated in 500 µL of liquid LB medium and incubated for 2 d at 28°C on a shaker (Shel Lab, Shaking Incubator SI4 model). Finally, 250 µL of the bacterial suspension were transferred to a new sterile tube containing 60% glycerol and stored at –80°C.

2.4. Characterization of bacterial strains

Bacterial strains were characterized based on the Gram staining technique. Morphological characterization of the colonies was performed based on edge, shape, colour, elevation, presence of odour, and other relevant traits. Bacterial cells were observed under an optical

microscope (Olympus BX53F2) at 100x magnification; images were acquired through a Canon EOS Rebel T6 camera and analysed with the EOS V.3 utility programme.

2.5. Screening for plant growth-promoting activities and salt tolerance

All strains were screened for the following PGP activities: indole (indol-3-acetic acid, IAA), EPS, siderophore, and organic acid production, P and K solubilization, ACC deaminase activity, and N fixation; their salt tolerance was also evaluated.

2.5.1. Salinity tolerance test

The modified protocol of Ayaz et al. (2022) was used for this test. LB solid medium supplemented with different concentrations (2%, 4%, 8%, 16% and 32%) of NaCl were inoculated with 5 µL of bacterial suspensions in triplicate. The growth of the bacteria was monitored daily, but only the bacteria that grew at any concentration of NaCl after five days were deemed positive for salinity tolerance.

2.5.2. Production of indole-3-acetic acid (IAA)

The indirect detection of IAA produced by bacteria was performed with a colorimetric assay using Salkowski's reagent (SKW; Lebrazi et al., 2020). Bacteria were grown for 3 to 5 days in TSB containing 200 µg mL^{-1} L-tryptophan. Then, 5 µL of the bacterial suspensions were added to 100 µL of TSB contained in each well of a 96-well plate and placed for 2 d at 27°C on an orbital shaker at 150 rpm. Two volumes of SKW reagent were then added to the plates and left in the dark for 30 min at room temperature. A pink colour indicates the presence of indoles.

2.5.3. Production of siderophores

To test siderophore production, agar plates with Chrome Azurol Sulfonate (CAS) were used following the protocol described by Schwyn and Neilands (1987) with slight modifications. According to Louden et al. (2011), a high concentration of hexadecyltrimethylammonium bromide (HDTMA) is toxic for Gram-positive bacteria, therefore, we followed the method of Payne (1994) where HDTMA was replaced by cetyltrimethylammonium bromide (CTAB). Siderophore activity was recorded after 7 days of incubation at 28°C based on the yellow to orange coloration of the colonies.

2.5.4. Organic acid production

To determine organic acid production (OAP), the protocol of Kumar et al. (2012) was adapted to a 96-well plate. MM9 medium was added with 1 g L^{-1} methyl orange and 1.5 % BactoAgar 150 µL of this medium and 5 µL of bacterial suspension were placed in each well and incubated at 26°C. The OAP test was considered positive when a pinkish-reddish colour was observed in the well.

2.5.5. Exopolysaccharide production

To determine the production of EPS, the protocol of Villota-Calvachi et al. (2022) was followed with slight modifications. LB medium supplemented with 3.6% sucrose and Bacto Agar was prepared and autoclaved; then 2.5 mL of 0.08% (w/v in distilled water) Congo red was added after filtration with a 0.22-µm polyvinylidene difluoride filter. A black colour is considered as positive and red/pink as negative.

2.5.6. P and K solubilization tests

Bacteria were inoculated on Pikovskaya's solid medium (PKV; Gupta and Pandey, 2019) modified for P solubilization and Alexandrov's medium (AXV; Hu et al., 2006) for K solubilization in a Petri dish with three 5-µL aliquots of each bacterial isolate. Bacteria were incubated at 28°C for 4, 7, and 12 d for the PKV assay and 3 d for the AXV assay. The development of halos (clear zones around colonies) is considered as P and K solubilizing activity.

2.5.7. Nitrogen fixation

The assay was performed as described by Cordova-Rodriguez et al. (2022). Liquid nitrogen-free (Nfb) medium added with 0.5% bromothymol blue (in 0.2 N KOH) was prepared and the pH adjusted to 6.8. The assay was conducted in 96-well plates with 150 µL of Nfb added with 5 µL of bacterial inoculum, incubated for 10 d at a temperature of 28°C on an orbital shaker at 150 rpm. A blue colour in the well indicates a positive response.

2.5.8. ACC deaminase activity

Using the protocol of Penrose and Glick (2003) ACC deaminase activity in the bacterial isolates was determined by their ability to utilize ACC (3 mM) instead of $(NH_4)_2SO_4$ as sole nitrogen source in DF (Dworkin and Foster, 1958) minimal salts medium. The inoculated plates were incubated at 28°C for three days and growth was monitored daily. Colonies growing on the plates were considered as ACC deaminase producers.

2.6. Bacterial identification

2.6.1. MALDI-TOF

Bacterial strain identification was performed using mass spectrometry MALDI (Matrix-Assisted Laser Desorption/Ionization) TOF/TOF (UltrafleXtreme, Bruker, Billerica, MA, USA), as reported by Novello et al. (2023). A fresh bacterial colony grown on TSA was spotted in triplicate on an MTP 384 target plate (Bruker Daltonics, Milan, Italy). The spot was covered with 70% formic acid (Sigma Aldrich, Burlington, MA, USA) and then with alpha-cyano-4-hydroxycinnamic acid (Bruker, Milan, Italy). The target plate was dried at room temperature until crystallization of the matrix. The mass spectra obtained for each bacterial strain were analysed using Biotyper software v. 2.0 (Bruker Daltonics, Milan, Italy). The identification was considered significant for species when the threshold was higher than 2, and for genus identification when the score was between 1.70 and 1.99, as recommended by the manufacturer.

2.6.2. Molecular identification of bacterial strain

Bacterial strain identification was performed by 16S rDNA sequencing as fully described in Gamalero et al. (2020). Briefly genomic DNA was extracted using NucleoSpin microbial DNA purification kit (Macherey- Nagel, M-Medical, Cornaredo, Milan) following the manufacturer's instructions, then16S rDNA PCR fragment was amplified using fD1 and rP2 modified primers, the PCR product was purified using Nucleo Spin Extract II kit (Macherey-Nagel) and then sequenced by BMR Genomics (Padua, Italy). Electropherograms were analyzed using Finch TV version 4.0 (Applied Biosystems, Milan, Italy) and DNA sequences were blasted against the National Center for Biotechnology Information (NCBI) database using BLASTn algorithm.

2.7. Arabidopsis growth conditions and treatments

Seeds of *Arabidopsis thaliana* ecotype Columbia (Col-0) were sterilized by placing them in a sealed container for 2 h in the presence of the gas produced by the reaction of commercial bleach mixed with 37% hydrochloric acid (50:1, v/v). Then, the seeds were dried under a sterile air flux for 2 h and stored at 4°C until use. Sterilized seeds were placed on agar plates containing MS 1X medium (Murashige and Skoog, 1962). Plates were placed vertically in a growth chamber under cool-white, fluorescent light with a photoperiod of 16/8 h light/darkness and a temperature of 21 ± 1°C.

Based on the determination of PGP activities, the bacterial strain having the highest score and identified as *Pseudomonas putida* (code A7), an endophytic bacterium from whole seeds of Pandela, was used as inoculant in *Arabidopsis*. A bacterial suspension at a density of 100,000 CFUs mL^{-1} was prepared by dilution in LB medium and poured on the surface of agar plates containing MS medium added with 100 mM NaCl

(saline medium, SM), or not (non-saline medium, NSM). Five-day old *Arabidopsis* seedlings were transferred to the plates and placed horizontally in a growth chamber (same conditions as above). After 12 days, seedling root architecture, *viz*. primary root (PR) length and number of lateral roots (LRs), was evaluated after scanning the plates and analysing the images with ImageJ software. Then, seedlings were collected, their fresh weight determined and frozen for biochemical, and gene expression analyses.

The presence of bacteria in seedlings was checked 14 days after inoculation with the standard inoculum (10^5 CFUs mL^{-1}). Control seedlings were treated with sterile distilled water. Plants (roots and rosettes separately) were harvested and surface-sterilized with 5% (v/v) sodium hypochlorite (1 min) followed by 70% (v/v) ethanol (1 min), then rinsed three-times in sterile distilled water. Sterilization efficiency was verified by placing the plant material on agar plates containing LB medium and incubating them for 48 h at 28°C. After removing the excess moisture, plant organs were macerated under sterile conditions. Approximately 5 to 10 µL of the homogenate were plated onto agar plates containing LB medium and incubated at 28°C. Plates were examined for bacterial colonies at 24, 48, and 72 h.

2.8. Biochemical features

2.8.1. Proline

Proline concentration was estimated following the method of Bates et al. (1973) with slight modifications. About 50 mg of fresh plant tissue were extracted in 1.2 mL 3% (w/v) aqueous 5-sulphosalicylic acid and then centrifuged at 9,500 x *g* for 15 min, while 50 mL of bacterial cultures were centrifuged and the pellet extracted overnight and then centrifuged in the same manner (Whatmore et al., 1990). A 500 mL-aliquot of supernatant was mixed with 2 mL of acid ninhydrin reagent and left at 100°C for 1 h. Samples were then cooled on ice. After 5 min, absorbance was read at 520 nm in a UV/Vis spectrophotometer (BioTeK). A standard curve was prepared using L-proline.

2.8.2. Photosynthetic pigments

About 50 mg of leaf tissue were ground and extracted with 10 volumes of 100% methanol. The extract was centrifuged at 4,500 x *g* for 20 min. After collecting the supernatant, chlorophylls (Chl*a*, Chl*b*, and Chl*a*+*b*) together with total xanthophylls and carotenoids (Cx+c) were spectrophotometrically determined by measuring absorbance at 665, 652, and 470 nm and applying the equations of Lichtenthaler (1987).

2.9. Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from rosettes (two separate extractions of 0.1 g FW each) as previously described (Ruiz et al., 2019). RNA yield and purity were checked by means of UV absorption spectra, whereas RNA integrity was determined by electrophoresis on agarose gel. DNA was removed using the TURBO DNA-free™ (Applied Biosystems, California, USA) from 5 to 10 µg aliquots of total RNA. First-strand cDNA was synthesized from 2 µg of the DNase-treated RNA by means of the High-Capacity cDNA Kit (Applied Biosystems) using random primers. The reaction mixture for the qPCR analysis was made in a final volume of 25 µL containing 10 ng of cDNA, 5 pmol of each primer, and 12.5 µL of the PowerUP SYBR Green PCR master mix (Thermo-Applied Biosystems) according to the manufacturer's instructions. *Adenine phosphoribosyltransferase1* (*AtAPT1*) was used as reference gene to normalize and estimate up- or downregulation of the target genes for all qPCR analyses. Sequences of *A. thaliana* genes were obtained from the NCBI database (Supplemental Table S1). qRT-PCR analyses were carried out with ARIANMAX 3200 for 2 min at 50°C, 30 sec at 95°C and then for 40 cycles as follows: 95°C for 3 s, 60°C for 30 s. Fold changes in RNA expression were estimated using threshold cycles and analysed by the comparative threshold cycle method, also known as the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Relative expression values were further processed using the QGene software (Simon 2003) to obtain normalized mean expression levels. Data are the means ± standard errors of two biological replicates and two technical replicates for each.

2.10. Statistical analysis

Each experiment on media with or without NaCl and/or A7 was randomly designed with four to six independent biological replicates (Petri dishes) per treatment, each containing 12 seedlings. For gene expression analyses, RNA extraction was done separately from two biological replicates, and two technical qPCR replicates were performed for each biological sample. Experiments were repeated twice.

To evaluate treatment effects, data were analysed using one-way ANOVA considering the four treatment groups (Control, A7, NaCl, and NaCl+A7). When significant differences were detected, Tukey's post-hoc test was applied for multiple comparisons. Homogeneity of variances was verified using Levene's test. Differences were considered significant at $p<0.05$. Statistical analyses were performed using INFOSTAT software (v. 2016).

3. Results

3.1. Morphological features and Gram-staining response of bacteria

After exposing quinoa seeds to chlorine gas, their sterility was checked. No microbial growth was observed, whereas non-sterilized control seeds showed abundant colony formation, thus confirming the effectiveness of the sterilization protocol in eliminating cultivable surface-associated bacteria. Bacterial colonies obtained after macerating surface-sterilized seeds were, therefore, cultivable endophytes.

The morphological characteristics of the colonies (colour, height, border shape, and other), the type of bacterial cells and their Gram reactivity are shown in Table 1. Colony borders ranged from complete to lobed and wavy, with most isolates showing entire (1–2) or lobed (3) edges. Colony colour varied from white to cream and yellow, while red and orange pigmentation was rarely observed. Most isolates showed convex to highly convex colonies (scores 3–4), with a few displaying flat or slightly spreading morphologies (scores 1–2). Colony shapes were predominantly circular (score 2) or irregular (score 4), whereas filamentous and rhizoid forms were uncommon. Additional features such as fluorescence or inhibitory halos were limited to the strain *Pseudomonas putida* A7. Microscopic examination confirmed the presence of both Gram-positive cocci and rods, and Gram-negative bacilli. According to their identification, isolates belonging to *Staphylococcus* spp. and *Bacillus* spp. were Gram-positive cocci and rods, respectively, whereas *Acinetobacter, Enterobacter*, and *Pseudomonas* species were Gram-negative rods or coccobacilli.

3.2. Salinity tolerance and PGP activities of bacterial strains

A total of 15 culturable bacterial strains were isolated from surface-sterilized whole quinoa seeds. The salt tolerance and the PGP activities of the 15 strains are shown in Table 2. All the isolates were able to tolerate 1%, 2%, and 4% NaCl; this trait indicates that the strains are halotolerant. Thirteen out of 15 strains (87%) grew in the presence of 16% NaCl and can be classified as moderately halophilic. Strains A1, A6, and A13 are extremely halophilic since they developed colonies on a medium added with 32% NaCl. Only one (A7) was fluorescent under UV light. The number of isolates able to solubilize P (A7) or K (A3 and A7) was very low. Similarly, only two isolates (A3 and A7) synthesized siderophores. The capacity to produce auxin (A3, A6, A7, A11, A14, B1) and organic acids (A4, A5, A7, A8, A13, A15) was more frequent as it was detected in six of the bacterial endophytes isolated from quinoa seeds. ACC deaminase activity, assessed qualitatively, was shared by nine strains, while EPS production was detected in 10 strains. On the contrary, only 2 out of 15 isolates (A3, A7) are nitrogen fixers. Taking

Table 1
Morphological and taxonomic characteristics of bacterial isolates obtained from quinoa seed-associated samples. Colony features were evaluated based on border, colour, height, shape, and additional traits, whereas bacterial cell morphology and Gram reaction were determined microscopically. Identification was confirmed by MALDI-TOF MS analysis.

			Colony					Bacteria	
Number	Code	MALDI ID	Border	Colour	Height	Shape	Other	Gram staining	Shape
1	A1	*Acinetobacter radioresistens*	1	3	4	2	0	-	4
2	A3	*Enterobacter cloacae*	2	6	0	4	0	-	1
3	A4	*Staphylococcus xylosus*	1	6	3	2	0	+	1
4	A5	*Bacillus sp*	3	3	2	4	0	+	1
5	A6	*Bacillus cereus*	1	6	3	2	0	+	4
6	A7	*Pseudomonas sp*	1	6	4	2	1	-	2
7	A8	*Staphylococcus xylosus*	3	3	6	5	0	+	1
8	A10	*Staphylococcus aureus*	1	3	1	1	0	+	1
9	A11	*Staphylococcus aureus*	1	3	4	2	0	+	1
10	A12	*Staphylococcus xylosus*	1	0	4	2	0	+	1
11	A13	*Staphylococcus xylosus*	1	3	3	1	0	+	1
12	A14	*Acinetobacter radioresistens*	2	3	2	4	0	-	4
13	B1	*Staphylococcus xylosus*	1	5	3	1	0	+	1
14	B5	*Staphylococcus xylosus*	5	3	0	3	0	+	1
15	B6	*Staphylococcus aureus*	2	3	1	1	0	+	1

Colony traits				Bacterial traits		
Border	Colour	Height	Shape	Others	Gram staining	Shape
0 ND	0 ND	0 ND	0 ND	0 ND		0 ND
1 Complete	1 Red	1 Flat	1 Punctate	1 Fluorescent	1 Positive	1 Coccus
2 Wavy	2 Yellow	2 Spread	2 circular	2 Forms inhibitory halo	2 Negative	2 Bacillus
3 Lobed	3 White	3 Convex	3 Filamentous	3 Callus type		3 Coccobacillus
4 Dented	4 Grey	4 Highly convex	4 Irregular	4 Irregular surface		4 Other
5 Filamentous	5 Orange	5 Bulging	5 Rhizoid			
6 Curly	6 Cream	6 Invaginated	6 Felling			
7 Other						

Table 2
Salt tolerance and plant growth-promoting (PGP) traits of bacterial isolates identified by MALDI-TOF MS. Bacterial growth was evaluated on nutrient agar supplemented with increasing concentrations of NaCl (1–32%). The presence (1) or absence (0) of specific PGP activities was recorded for phosphate and potassium solubilisation (Psol and Ksol), siderophore production (SID), organic acid production (OAP), 1-aminocyclopropane-1-carboxylate deaminase activity (ACC), indole-3-acetic acid production (IAA), exopolysaccharide formation (EPS), and nitrogen fixation (Nfb). Fluorescence under UV light was also noted. Score represents the total number of positive traits for each isolate.

Number	Code	MALDI ID	NaCl					P-sol	K-sol	SID	OAP	ACC	IAA	EPS	Nfb	Fluorescence	SCORE
			1%	2%	4%	16%	32%										
1	A1	*Acinetobacter radioresistens*	1	1	1	1	1	0	0	0	0	1	0	1	0	0	4
2	A3	*Enterobacter cloacae*	1	1	1	0	0	0	1	1	0	1	1	1	1	0	6
3	A4	*Staphylococcus xylosus*	1	1	1	1	0	0	0	0	1	0	0	0	0	0	2
4	A5	*Bacillus sp.*	1	1	1	1	0	0	0	0	1	1	0	1	0	0	4
5	A6	*Bacillus cereus*	1	1	1	1	1	0	0	0	0	1	1	1	0	0	5
6	A7	*Pseudomonas sp.*	1	1	1	0	0	1	1	1	1	1	1	0	1	1	**7**
7	A8	*Staphylococcus xylosus*	1	1	1	1	0	0	0	0	1	0	0	0	0	0	2
8	A10	*Staphylococcus aureus*	1	1	1	1	0	0	0	0	0	0	0	1	0	0	2
9	A11	*Staphylococcus aureus*	1	1	1	1	0	0	0	0	0	0	1	1	0	0	3
10	A12	*Staphylococcus xylosus*	1	1	1	1	0	0	0	0	0	0	0	1	0	0	2
11	A13	*Staphylococcus xylosus*	1	1	1	1	1	0	0	0	0	0	0	1	0	0	3
12	A14	*Acinetobacter radioresistens*	1	1	1	1	0	0	0	0	1	1	1	1	0	0	5
13	B1	*Staphylococcus xylosus*	1	1	1	1	0	0	0	0	0	1	1	1	0	0	4
14	B5	*Staphylococcus xylosus*	1	1	1	1	0	0	0	0	0	1	0	0	0	0	2
15	B6	*Staphylococcus aureus*	1	1	1	1	0	0	0	0	0	1	0	0	0	0	2

into consideration all these physiological traits, strain A7, followed by A3, obtained the highest scores for PGP activities.

3.3. Bacterial identification by Maldi-ToF and 16S RNA sequencing

Bacterial strains isolated from the inner tissues of quinoa seeds were identified by MALDI-TOF (Table 2). Of the 15 bacterial strains, 40% belong to the species *Staphylococcus xylosus*, 20% to *Staphylococcus aureus*, 13% to *Acinetobacter radioresistens*, and 6.6% to *Enterobacter cloacae* and *Bacillus cereus.* The remaining bacterial strains were identified at the genus level as *Pseudomonas* (6.6%) and *Bacillus* (6.6%). The identification of *Pseudomonas* A7, which obtained the highest score for its PGP features, was confirmed by molecular analysis. This microorganism was identified as *Pseudomonas putida* through comparison of its16S rDNA with all sequences available in the NCBI database. In particular, the sequence had 100% coverage, BLAST identity 99.81% and E value 0.00E+00 compared to *P. putida* strain BGRI_EBC_SS23-S10 accession OR166232.1. The bacterial 16S rDNA sequence is available at the NCBI database as GenBank accession PX459935 (submission 15684638). Details are shown in Supplemental Table S2.

3.4. Proline content in P. putida strain A7

Proline concentration in bacterial cells (two inoculum densities) six

days after transfer to fresh MS medium added with 100 mM NaCl increased significantly, reaching *ca.* 230-380 μmol g^{-1} FW or *ca.* 9-50 μmol mL^{-1} (Table 3).

3.5. Effects of strain A7 on Arabidopsis growth and root architecture

No bacterial colonies were detected in non-inoculated control plants, confirming the effectiveness of surface sterilization. In contrast, plates from tissues of inoculated plants showed visible bacterial colonies after 48–72 h, indicating successful internal colonization of *A. thaliana* by the inoculated A7 strain.

In the absence of the bacterial strain, 100 mM NaCl dramatically reduced the fresh weight of *Arabidopsis* seedlings (Fig. 1 A). On NSM, A7 alone inhibited plant growth, but restored it to control levels under salt stress conditions. Salinity reduced both PR length (Fig. 1B) and number of LRs (Fig. 1C, D). When co-cultured with strain A7, PR length of seedlings was strongly inhibited both on NSM and SM. By contrast, the number of LRs was enhanced compared with the control under non-saline conditions and restored to control values in the presence of salt.

Different from strain A7, a highly salt-tolerant strain of *Leclercia adecarboxylata* isolated from quinoa seeds, which also exhibited multiple PGP activities (P solubilization, OAP, ACC deaminase activity, and IAA and EPS production), inhibited growth both under saline and non-saline conditions (data not shown).

3.6. Effects of strain A7 on biochemical responses

Proline was enhanced *ca.* four-fold by salt in the absence of bacteria (Fig. 2). When co-cultured with A7 without NaCl, proline levels were increased *ca.* two-fold relative to non-inoculated controls, but under saline conditions they were not significantly affected by the presence of A7.

Salinity reduced both Chl*a*, and Chl*b* concentrations (Fig. 3A-C). In inoculated seedlings grown on NSM, Chl*b* was slightly but significantly less than in controls (Fig. 3B), while on SM, A7 restored Chl*a* concentrations to control levels and enhanced Chl*b* relative to salt alone. Total carotenoids were not affected either by salt or by the presence of A7 (Fig. 3D).

3.7. Gene expression in response to salinity and inoculation with A7

Transcript levels of genes involved in growth, photosynthesis, nitrate transport, N sensing, ion homeostasis, proline metabolism, redox status, and auxin transport and signaling (Table 4) were evaluated in *Arabidopsis* seedlings 12 days after transfer to NSM or SM, in the presence or absence of the selected bacterial isolate (A7).

As shown in Figs. 4, 5, and 6, the analysed genes could be subdivided into the following three categories:

a) genes whose expression was modulated by salt stress - many of the analysed genes, namely *AtHEMA, AtCYCD2, AtNRT1.1, AtANR1, AtASA1, AtGRX3* and *4, AtNHX, AtSOS1,* and *AtProDH1* and *2*. Only *AtHKT1*, and *AtP5CS* were upregulated under saline conditions.

Table 3
Proline content in *Pseudomonas putida* strain A7 after six days of growth on MS medium added with 0 or 100 mM NaCl. Values are means ± standard error. Different letters indicate significant ($p<0.05$) differences. n.d., not detectable.

		Proline content	
Inoculum size (CFUs mL^{-1})	NaCl (mM)	μmol g^{-1} FW	μmol mL^{-1}
10^5	0	n.d.	n.d.
	100	234.0 ± 55.1^{b}	9.3 ± 2.2 ^{c}
10^{14}	0	97.9 ± 3.7^{c}	31. 3 ± 1.2 ^{b}
	100	382.2 ± 21.9^{a}	49.5 ± 2.8 ^{a}

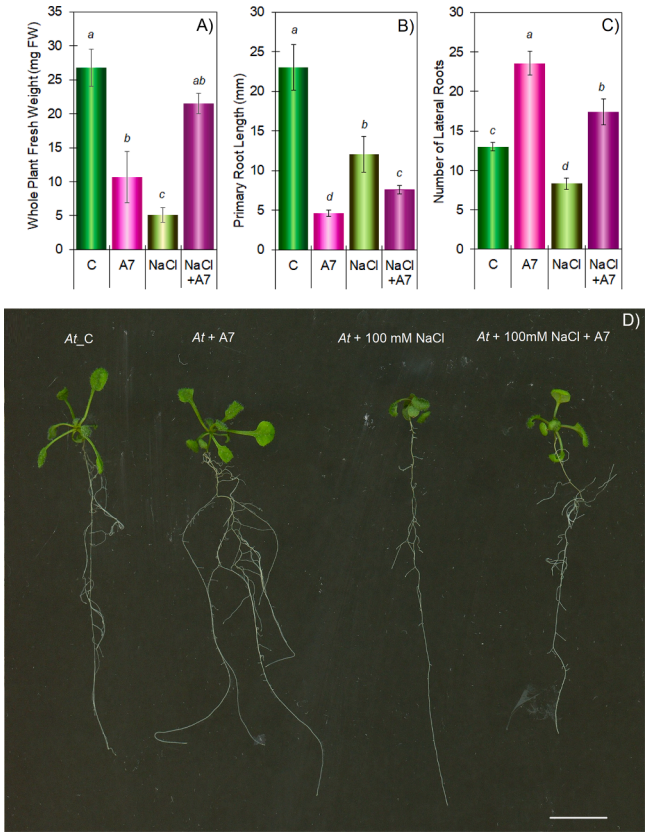


Fig. 1. Growth and morphology of *Arabidopsis* seedlings inoculated or not with strain A7 and grown for 12 days in axenic culture on non-saline or saline (NaCl) medium. (A) Fresh weight of whole plants. (B) Primary root length. (C) Number of lateral roots per primary root. (D) Photograph of seedlings grown under the different culture conditions. Bar indicates 10 mm. C, control. Different letters indicate statistically significant differences between treatment means ($p<0.05$) according to one-way ANOVA followed by Tukey's post-hoc test.

b) genes whose expression was modulated by inoculation with strain A7 in the absence of salt - co-culture with A7 on NSM modulated expression of *AtCYCD2, AtHEMA, AtNRT1.1, AtANR1, AtASA1, AtTIR, AtHKT, AtNHX,* and *AtSOS1*. Except for *AtANR1* and *AtASA*, all the genes were downregulated by bacterial inoculation.

c) genes whose expression was modulated by inoculation with strain A7 in the presence of salt - compared to salt-stressed plants without bacteria, co-culture with A7 enhanced transcript levels of several genes *(AtCYCD2, AtHEMA, AtNRT2.1, AtANR1, AtGRX3* and *4, AtSOS1,* and *AtNHX1),* generally restoring them to control values when they were downregulated by salt.

4. Discussion

4.1. Quinoa seed endophytic bacteria are halotolerant and exhibit various PGP activities

Isolation of halotolerant and halophilic bacterial endophytes from quinoa seeds is consistent with the classification of the soil of this geographical region as a saline one (Luzio, 2010) and highlights the adaptability of this plant species and its associated microbiome to harsh environmental conditions. The predominance of *Staphylococcus* spp., particularly the species *S. xylosus* and *S. aureus* (40 and 20%, respectively), aligns with their well-known tolerance to osmotic stress, which would facilitate their survival in saline environments, where osmotic stress typically limits competition with salt-sensitive bacterial species. In fact, members of this genus are recognized as halotolerant or even

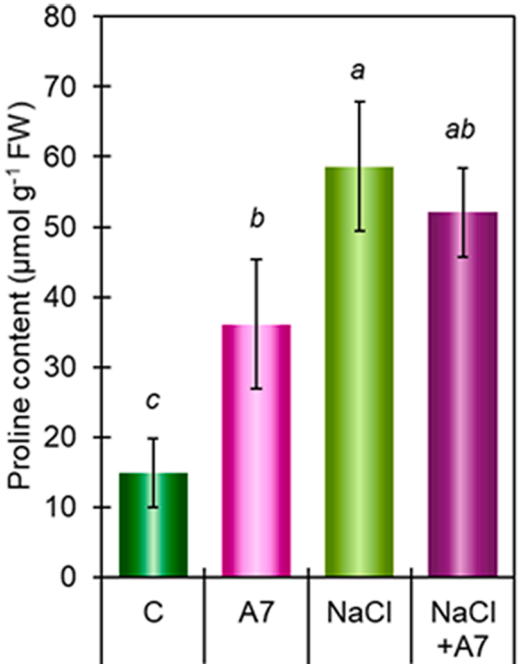


Fig. 2. Proline content in *Arabidopsis* seedlings inoculated or not with strain A7 and grown for 12 days in axenic culture on non-saline or saline (NaCl) medium. C, control. Different letters indicate statistically significant differences between treatment means ($p<0.05$) according to one-way ANOVA followed by Tukey's post-hoc test.

halophilic microorganisms, capable of thriving in high-salt environments, especially in food, due to efficient osmoregulatory mechanisms, such as the accumulation of compatible solutes (Parfentjev and Catelli, 1964; Zeng et al., 2017). Genomic analyses confirmed that *S. aureus* possesses multiple osmotolerance mechanisms—including duplicated genes encoding compatible solute transporters and Na^+/H^+ antiporters—enabling its survival in hyperosmotic environments (Kumar et al., 2021). Similarly, *S. xylosus* strains have been shown to tolerate up to 10% NaCl and remain metabolically active at low pH and temperature, confirming their broad ecological plasticity (Zeng et al., 2017). The detection of *S. aureus* and *S. xylosus* as endophytes in quinoa seeds requires careful consideration. Although the former is a well-known opportunistic pathogen capable of causing a wide range of infections in humans and animals, this bacterial species has been isolated directly from environmental samples such as soil (Singh and Kumari, 2023), the leaf internal tissue of *Azadirachta indica* (neem) (Singh et al., 2017), inside fruit tissues (Agarwal and Sheikh, 2025), and finally, in the endosphere of *Anadenanthera colubrina*, an endemic tree of South America whose parts are used by local populations for the treatment of various diseases and in indigenous rituals (Alibrandi et al., 2018). The detection of *S. aureus* in association with plants does not necessarily imply true and active colonization or endophytic lifestyle; such occurrences may reflect transient environmental reservoirs or vectors linked to soil, irrigation water, or agricultural handling. Even more, it is important to distinguish between clinically relevant strains and environmental representatives belonging to the same species, since their ecological roles may differ substantially from those of clinical strains. The strains identified in our study were not subjected to virulence or antibiotic resistance profiling, so that no conclusions can be drawn regarding their potential pathogenicity. Importantly, strains assigned to *Staphylococcus* spp. were not selected for plant inoculation experiments, and no agricultural application of these isolates is proposed. On the other hand, *S. xylosus* has long been regarded as a non-pathogenic coagulase-negative species, commonly isolated from the skin and mucosa of humans and animals, as well as from fermented food products where it is often intentionally used as a starter culture. Although several isolates exhibited *in vitro* PGP-related traits, their ecological role within quinoa seeds remains to be functionally demonstrated. Their possible and under-investigated adaptive features, including salt tolerance, biofilm formation, and potential antibiotic resistance, underscore the need for integrated monitoring within the One Health perspective, particularly for edible or seed crops.

To our knowledge, only one recent study (Maestro-Gaitán et al., 2025) has characterized the core endophytic bacteria of quinoa seeds. Their results revealed a high abundance of members of the Micrococcaceae family, particularly the genus *Pseudarthrobacter*, followed by representatives of Gammaproteobacteria and Bacilli. Unfortunately, taxonomic identification in this study was not reported at the species level, thus not allowing a comparison with our data, showing *Staphylococcus xylosus* and *S. aureus* as the dominant species among culturable bacterial seed endophytes. Conversely, members of the Pseudomonadaceae family and of class Gamma- Proteobacteria were detected in both studies, suggesting that at least part of the quinoa seed endophytic community may be conserved across different genotypes and environmental conditions.

In our study, several Gram-negative isolates, including *Enterobacter cloacae A3, Acinetobacter radioresistens* A14, and *Pseudomonas* sp. *A7*, also exhibited considerable halotolerance, an uncommon trait among Gram-negative bacteria. The former two strains were able to synthesize EPS, a key adaptive mechanism in saline environments. These biopolymers form a hydrated matrix surrounding the bacterial cells, providing both physical and chemical protection against osmotic/saline stress and facilitating a more stable beneficial plant–microbe interaction (Bhagat et al., 2021). This physiological feature was shared by seven other Gram-positive strains isolated from quinoa seeds. In strain A7 of *P. putida*, proline content increased significantly in the presence of 100 mM NaCl, and the maximum concentration reached was comparable to that reported for *Bacillus subtilis* (strain NCIB 1650) exposed to 400 mM NaCl (Whatmore et al., 1990) and *Enterobacter cloacae* (BHUAS1) grown in the presence of 500 mM NaCl (Kumar and Prasad, 2025). Both studies corroborated the osmoprotectant role of proline in bacteria, allowing growth under salt/osmotic stress conditions. Proline accumulation likely contributed to the salt tolerance of strain A7.

The number of bacterial strains able to synthesize siderophores, as well as to solubilize P and K, was very low. In our dataset, only 2 out of 20 isolated strains showed siderophore production under standard conditions. This finding is intriguing given the high iron content reported for quinoa seeds, with concentrations up to 81 mg/kg of seed dry weight (Prado et al., 2014). One possible explanation is that the relatively high internal iron levels may suppress siderophore biosynthesis *via* feedback inhibition mechanisms (Venturi et al., 1995). However, total iron content does not necessarily reflect its bioavailability to endophytes. Iron within plant tissues is often bound to phytates or other chelating compounds, which can significantly limit its accessibility to microorganisms (Melini and Melini, 2021). Similarly, the amount of P and K inside quinoa seeds has been estimated at 4,120 and 9,520 mg kg^{-1} seed dry weight, respectively (Prado et al., 2014) and these concentrations are quite high compared to cereals, such as rice, wheat, corn, barley, and oat. The low abundance of K and P solubilizers might be related to the chemical forms in which these nutrients are stored within the seed. While P in seeds is predominantly found as phytic acid that is not readily available to microorganisms, K is typically stored in organic or exchangeable forms (Ragel et al., 2019) that may not require microbial solubilization for access. This suggests a reduced ecological pressure for maintaining a large community of K-solubilizing endophytes. Nevertheless, as with iron, the distinction between total nutrient content, the capability to synthesize phytase and microbial bioavailability is critical, together with the variability of the concentration of these minerals inside quinoa seeds. Data regarding the relationship between seed iron, K, and P availability and siderophore production as well as the solubilization capacity of endophytic bacterial communities

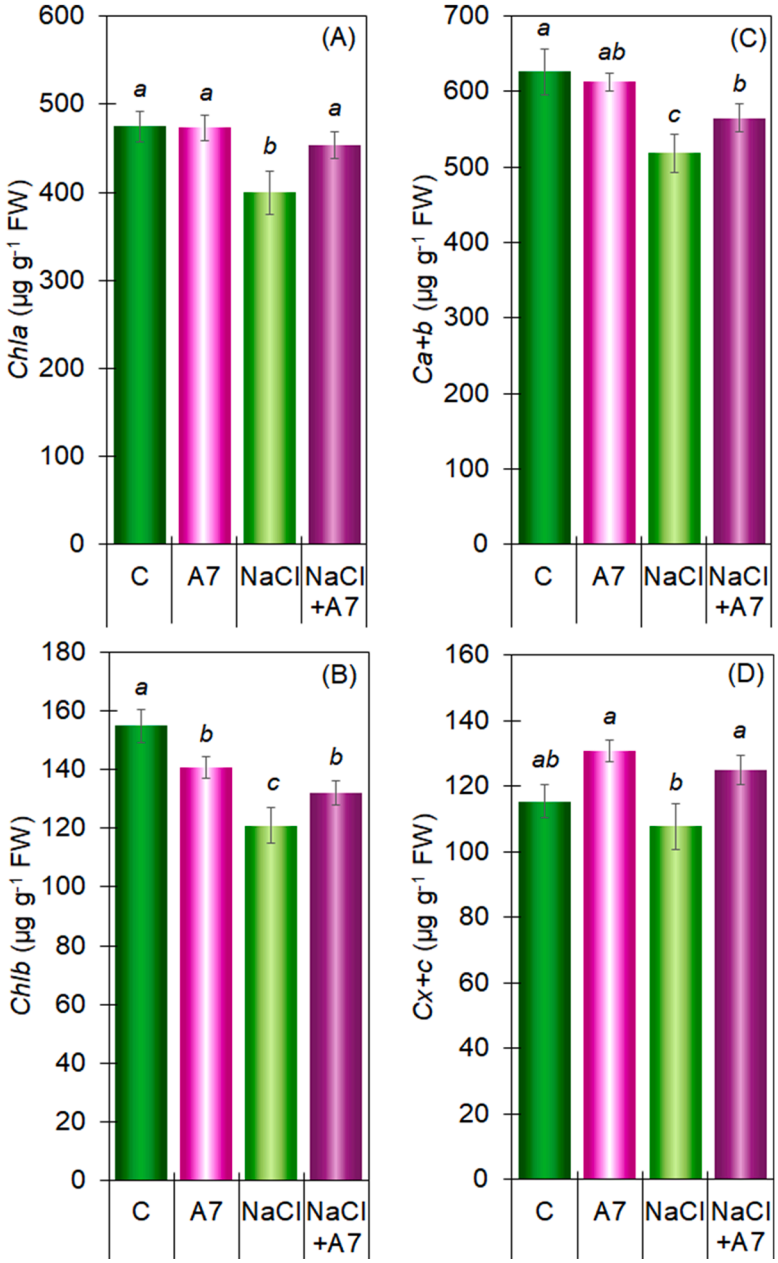


Fig. 3. Chlorophyll content in *Arabidopsis* seedlings inoculated or not with strain A7 and grown for 12 days in axenic culture on non-saline or saline (NaCl) medium. C, control. Different letters indicate statistically significant differences between treatment means ($p<0.05$) according to one-way ANOVA followed by Tukey's post-hoc test.

in quinoa are lacking. These gaps highlight the need for further targeted studies to clarify the ecological and physiological factors influencing siderophore-mediated iron acquisition in the seed microbiome.

Interestingly, a high proportion of quinoa seed endophytes were able to produce ACC deaminase. This enzyme degrades ACC, the immediate precursor of ethylene, a plant hormone involved in stress responses and seed germination. Although ethylene in dormant seeds is generally produced at low levels, it can quickly increase upon imbibition or under abiotic stress, such as salinity (Zeng et al., 2024). The quinoa used in this study was cultivated on saline soils in the Chilean Andes, and the variety is known to be salt-tolerant. These conditions may select endophytic bacteria capable of modulating ethylene levels to mitigate salt-induced stress during germination. The high frequency of ACC deaminase-producing bacteria may, therefore, reflect an ecological adaptation aimed at promoting seed vigor and early seedling establishment under adverse environmental conditions. However, direct measurements of ethylene or ACC content in this variety of quinoa seeds are still lacking, and further studies are needed to clarify this potential relationship.

Overall, the ability of these strains to combine salt tolerance with PGP traits, such as IAA, siderophore, and organic acid production as well as ACC deaminase activity, may contribute to quinoa's resilience under saline conditions. Interestingly, the strains isolated from quinoa seeds were assigned to genera such as *Bacillus, Pseudomonas*, and *Enterobacter* that are frequently described in the literature as having PGP and salt stress mitigating effects (Xu et al., 2014; Qin et al., 2016; Lastochkina et al., 2017; Pal et al. 2021). Strain A7, tolerating up to 4% NaCl and expressing all (except EPS production) of the tested PGP traits was identified as a *Pseudomonas* species by MALDI. Given that it obtained the highest score for plant beneficial features, we proceeded with 16S rDNA sequencing, thus identifying it as *Pseudomonas putida*.

Table 4
List of genes analysed by qRT-PCR in *Arabidopsis thaliana* seedlings.

Selected gene	Name	Function
AtAPT1	*Adenine phosphoribosyl transferase 1*	Purine metabolism
AtCYCD2	*CyclinD2*	Cell cycle-related
AtHEMA	*Glutamyl-tRNA reductase*	Chlorophyll biosynthesis
AtNRT1.1; AtNRT2.1	*Nitrate transporter 1.1 and 2.1*	Nitrate transport
AtANR1	*Arabidopsis nitrate regulated1*	Nitrogen sensing
AtHKT1	*High-affinity K^+ transporter1*	Ion homeostasis
AtSOS1	*Salt Overly Sensitive1*	Salt stress signaling
AtNHX1	*Na^+/H^+ exchanger1*	Ion homeostasis
AtP5CS	*Δ1-Pyrroline-5-carboxylate synthase*	Proline biosynthesis
AtProDH2	*Proline dehydrogenase*	Proline catabolism
AtGRX3; AtGRX4	*Glutaredoxin 3 and 4*	Oxidoreductases
AtASA	*Anthranilate synthase alpha subunit 1*	Auxin biosynthesis
AtTIR	*Transport Inhibitor Response 1*	Auxin receptor

4.2. Strain A7 enhances root branching and mitigates salt stress in *Arabidopsis*

Arabidopsis seedlings were co-cultured in axenic culture, under saline and non-saline conditions, with A7, the quinoa seed-derived *Pseudomonas putida* strain. Based on its halotolerance and multiple PGP activities, A7 was a good candidate (not necessarily the best) for alleviating salt stress-induced growth reduction and so it was decided to continue work with this strain. Results obtained with the *Leclercia adenocarboxylata* strain suggest that not all halotolerant bacteria with multiple PGP activities have beneficial effects when co-cultured with *Arabidopsis* under *in-vitro* conditions. After 12 days of inoculation with A7, morphological, biochemical, and gene expression analyses were conducted on the seedlings. The presence of bacteria inside the plant was also verified.

Our results revealed a negative effect on growth (biomass and root length) exerted by A7 under non-saline conditions. In an earlier study, *Arabidopsis* seedlings treated with quinoa seed hull powder (QHP) used as a biostimulant showed reduced growth in the absence or with the lowest concentration of NaCl, whereas under higher salinity conditions, the optimal dose of QHP improved shoot, and root biomass and primary root length compared with controls (Ruiz et al., 2025). Other studies using priming agents as biostimulants revealed that these inhibited growth under normal conditions, but significantly increased it under salt stress, confirming the notion that priming can retard growth under optimal conditions and promote it under stressful ones. Chu et al. (2019) tested a rhizospheric *Pseudomonas* strain (PS01) on *A. thaliana* seed germination under saline and non-saline conditions. Its effect was examined on MS medium with or without 150 mM NaCl. They observed that germination rate in the presence of NaCl was significantly increased in PS01-inoculated seeds when compared to the control. However, no significant difference in germination rate was observed between inoculated and non-inoculated seeds on control medium, suggesting that the PS01 treatment had no effect in normal conditions. It would appear that, like other biostimulants, PGPB are effective under stressful conditions (at least in artificial systems), whereas *Arabidopsis* seedlings on MS medium are in optimal germination/growth conditions. Thus, activities such as K and P solubilization, are probably not relevant, whereas reducing stress-induced ethylene production (via ACC deaminase) under stress can be. Auxin and ACC deaminase are, on the other hand, involved in LR formation (see below) and strain A7 promoted this process both under saline and non-saline conditions.

While on NSM A7 inhibited plant growth, it restored whole plant FW to control levels (no bacteria, no salt) under salt stress conditions. These results align with numerous reports on the salt stress-alleviating effect of inoculation with rhizobacteria in several crop species (lettuce, maize, peanut, chickpea, rice, wheat; Zahra, 2024 and references therein). Chu et al. (2019) isolated a specific *Pseudomonas* strain from the rhizosphere of maize plants growing in Vietnam. The strain was identified as a member of the *Pseudomonas putida* subclade. *Arabidopsis* seeds pre-inoculated with this strain survived under highly saline conditions (up to 225 mM NaCl), while non-inoculated plants did not. The transcriptional levels of genes related to stress tolerance were modified in inoculated plants compared to non-inoculated controls; *LOX2* (involved in jasmonate biosynthesis) was up-regulated, while the ascorbate

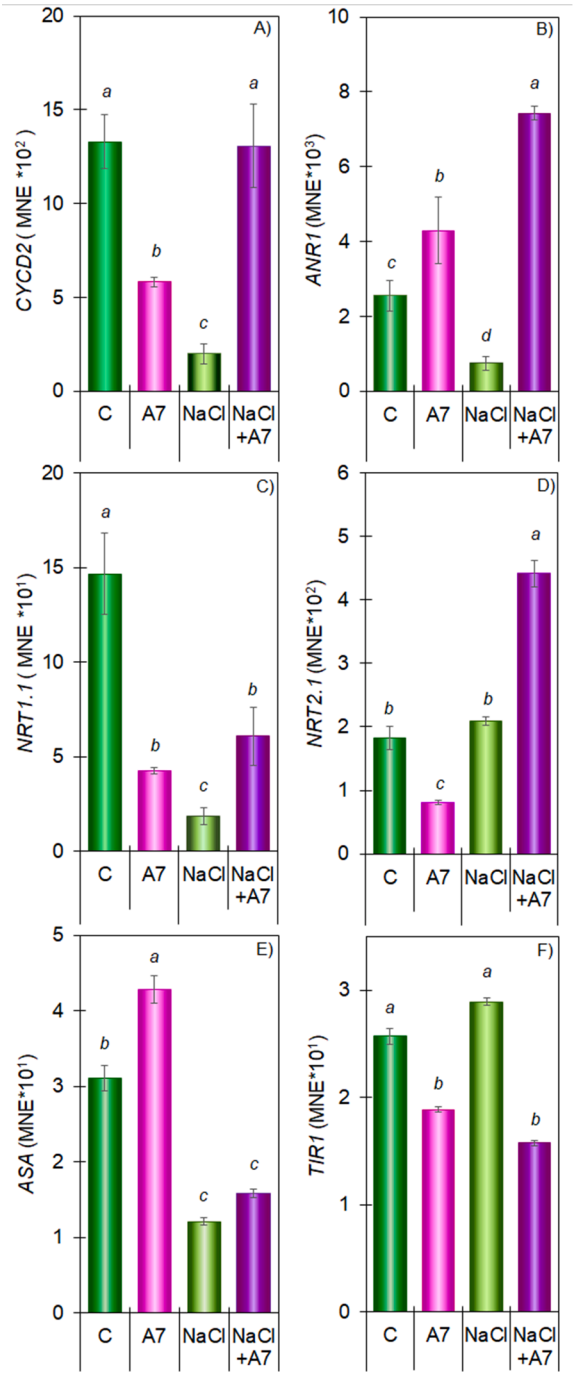


Fig. 4. Transcript abundance of *AtCYCD2*, *AtANR1*, *AtNRT1.1*, *AtNRT2.1*, *AtASA1*, and *AtTIR1* in 12- day old *Arabidopsis* seedlings inoculated or not with strain A7 and grown on non-saline or saline (NaCl) medium. C, control. Different letters indicate statistically significant differences between treatment means ($p<0.05$) according to one-way ANOVA followed by Tukey's post-hoc test.

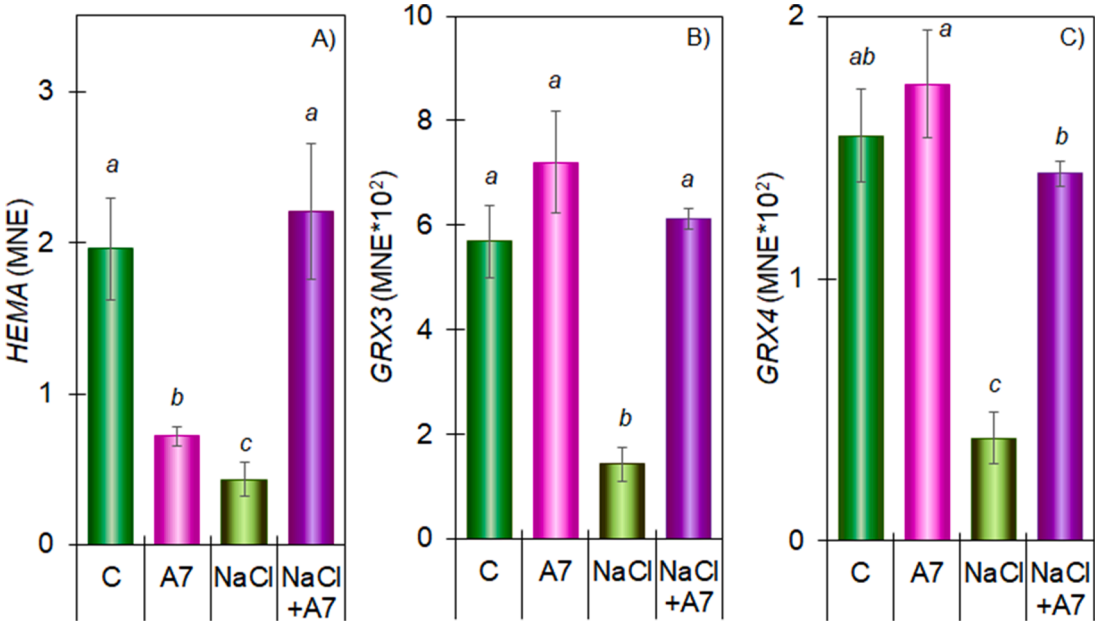


Fig. 5. Transcript abundance of *AtHEMA*, *AtGRX3*, and *AtGRX4* in 12-day old *Arabidopsis* seedlings inoculated or not with strain A7 and grown on non-saline or saline (NaCl) medium. C, control. Different letters indicate statistically significant differences between treatment means ($p<0.05$) according to one-way ANOVA followed by Tukey's post-hoc test.

peroxidase gene *APX2* and the glyoxalase gene *GLY17* were down-regulated. *P. putida* was already reported to mitigate the detrimental effects of salinity on germination and growth in *Brassica napus* (Jalili et al., 2009) and cotton (Egamberdieva et al., 2015).

Similar reports on the salt stress-mitigating effects of bacterial endophytes from aerial parts of plants are scarce (Mahgoub et al., 2021; Wael et al., 2024). Even less is known about seed endophytes and salinity tolerance. Seed endophytic bacteria with PGP traits, namely the ability to solubilize minerals, and fix N, were shown to be involved in the establishment of giant cardon cactus (*Pachycereus pringlei*) on barren rocks (Puente et al., 2009). A seed-derived *Bacillus subtilis* (HYT-12-1) with ACC deaminase activity was shown to improve growth in tomato seedlings (Xu et al., 2014). In *Nicotiana tabacum*, the phytotoxic effects of Cd were mitigated by seed-borne endophytic bacteria (*Pseudomonas* sp., and *Enterobacter* sp.) collected from plants grown on Cd- and zinc-enriched soil (Mastretta et al., 2009). The stress-mitigating effect of A7 in terms of growth is likely associated with its PGP activities described in the previous section, especially ACC deaminase. In fact, this enzyme catalyses the conversion of ACC (the precursor of ethylene biosynthesis) to ammonia and α-ketobutyrate, resulting in lesser production of the growth-inhibiting stress hormone ethylene (Glick et al., 2007). Orozco-Mosqueda et al. (2020) emphasized the importance of endophytic and rhizospheric PGPB with ACC deaminase activity in promoting plant growth under salt stress and suggested that such microbes could be used as bioinoculants to mitigate the negative impact of salinity in agricultural soils. The ability of PGPR to solubilize insoluble P and fix atmospheric N asymbiotically is likewise well documented and was also demonstrated in strain A7. The siderophores produced by PGPB, including A7, would make iron more easily accessible to plants. Thus, phosphate, and iron solubilization and N fixation/ammonia production would increase the supply of essential nutrients whose uptake by plants can be limited by the presence of NaCl, leading to improved growth/salt tolerance. Another PGP activity of strain A7, *viz.* the production of auxin, could also benefit the plant both in terms of growth and salt stress alleviation (Javid et al., 2011). The secretion of other phytohormones (e.g. cytokinins, gibberellins) by PGPR has been documented (Qin et al., 2016 and refs therein), as well as the production of gibberellins by endophytic bacteria isolated from leaves, stems, and seeds of halotolerant species (Wael et al., 2024). The seed endophytic bacterium *Priestia megaterium PH3* was reported to alleviate salt stress during peanut seed germination through a multifaceted approach involving, among others, the upregulation of genes involved in abscisic acid (ABA) catabolism and gibberellin synthesis (Li et al., 2025).

Primary root length was strongly inhibited by salt and by co-culture with A7 both under non-saline and saline conditions. Interestingly, however, the number of LRs was notably enhanced in inoculated plants under non-saline conditions and restored to control values in the presence of salt. The developmental plasticity of the root system is regarded as a crucial adaptive trait allowing plants to cope with abiotic stress factors, such as salinity (Lavenus et al., 2013; Zou et al., 2021) and variations in nutrient (nitrate, phosphate) availability (Zhang and Forde, 2000; Pérez-Torres et al., 2008). Root branching, i.e., LR formation, is a primary component of the root's response to its environment, a process that is tightly linked to auxin, although crosstalk with other plant growth regulators (e.g., cytokinins, brassinosteroids) has been proposed (Lavenus et al., 2013; Altamura et al., 2023). In fact, the plant's microbiota (PGPB, mycorrhiza) plays an important role in remodelling root architecture (Gonin et al., 2023), mainly through auxin production. High auxin levels can also induce the synthesis of ACC in plants, leading to a boost in ethylene production. A portion of this ACC is exuded from roots and seeds, taken up by bacteria, and metabolized by ACC deaminase. Thus, ACC deaminase–producing bacteria alleviate ethylene-mediated inhibition of growth (Gamalero and Glick, 2011) and LR formation (Lewis et al., 2011). Moreover, when ACC deaminase is present, auxin-dependent gene expression is not suppressed, enabling IAA to stimulate cell proliferation and organogenesis. Inoculation of *Arabidopsis* with the *P. putida* isolate A7, able to produce both IAA and ACC deaminase, resulted in reduced primary root elongation together with increased branching, reflecting the interplay between auxin and ethylene, with consequences on root system architecture.

Endophytic bacteria have been shown to regulate the expression of genes in host plants, such as those encoding antioxidant enzymes and other genes involved in responses to salinity stress (reviewed by Ali et al. 2022). For example, rice plants inoculated with a Cd-tolerant endophytic isolate from rice seeds accumulated lower amounts of this metal by downregulating expression of Cd transporters (Zhou et al., 2020). In

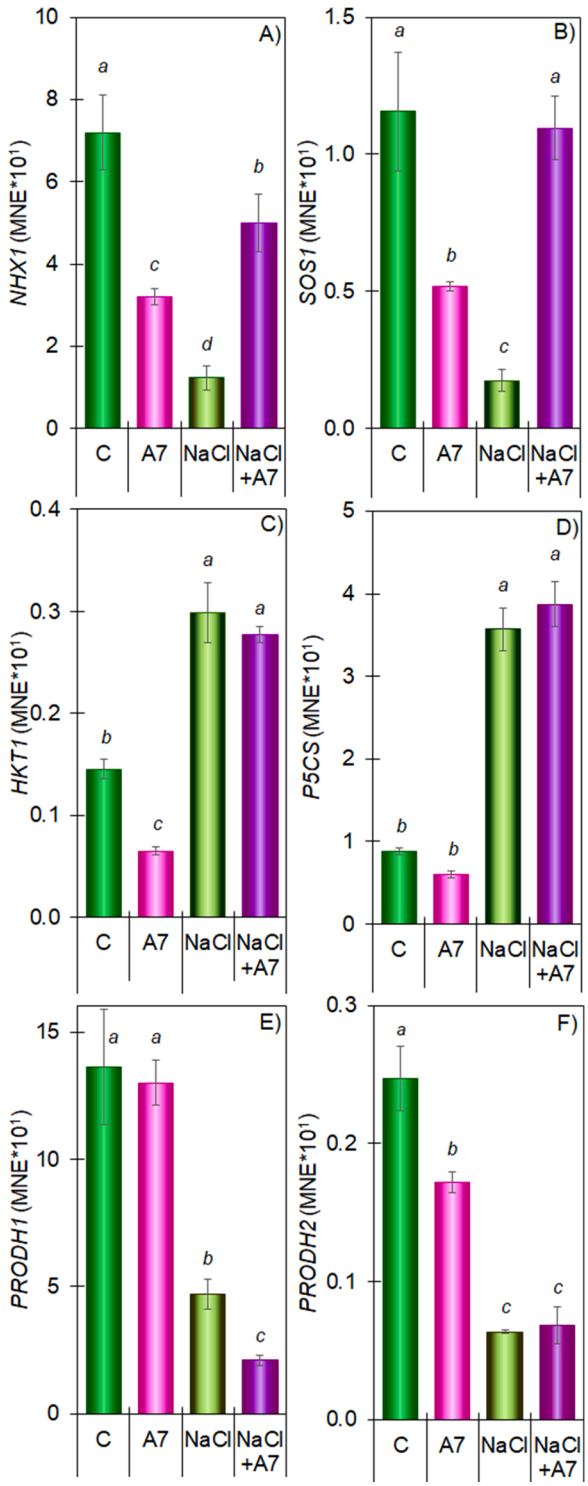


Fig. 6. Transcript abundance of *AtNHX1*, *AtSOS1*, *AtHKT1*, *AtP5CS*, *AtProDH1*, and *AtProDH2* in 12- day old *Arabidopsis* seedlings inoculated or not with strain A7 and grown on non-saline or saline (NaCl) medium. C, control. Different letters indicate statistically significant differences between treatment means ($p<0.05$) according to one-way ANOVA followed by Tukey's post-hoc test.

this study, we analysed 12 genes belonging to several different functional categories, *i.e.*, cell division, photosynthesis, nitrate and Na^+ ion transporters, proline metabolism, N sensing, redox status, and auxin biosynthesis and signaling.

The cyclin D2-encoding gene *AtCYCD2* was down-regulated by salt in accord with the observed salt-induced growth reduction (Fig. 4A). In fact, transcription of many cyclin genes is downregulated by salt/osmotic stress (Zhao et al., 2014; Ruiz et al., 2017). In seedlings co-cultured with A7, the expression pattern of this gene correlated with growth responses. Thus, growth was inhibited and *AtCYCD2* downregulated by co-culture with A7 on NSM, but growth and *AtCYCD2* transcript levels were restored to control levels under saline conditions (Fig. 4A). D-type cyclins (CYCD) are regulators of the cell cycle G1-to-S phase transition and are, therefore, likely to play a role during *de novo* formation of LRs from pericycle cells (Sanz et al., 2011). Enhanced LR number induced by A7 on SM could also relate to this upregulation of *AtCYCD2*. ARABIDOPSIS NITRATE REGULATED 1 (ANR1) is a transcription factor, functioning downstream of NRT1.1, involved in nitrogen sensing. The Arabidopsis *ANR1* knockout mutant is insensitive to ABA, salt, and osmotic stress during seed germination and early seedling development, whereas *ANR1*-overexpressing lines are hypersensitive (Lin et al., 2020). Herein we showed that *AtANR1* transcript abundance was enhanced by the presence of A7, both on NSM and, to an even greater extent, on SM, aligning with the effect it had on *AtNRT2.1* (Fig. 4B). ANR1 has also been involved in LR elongation under high nitrate conditions; nitrate-induced Ca^{2+}-ANR1 signaling promotes LR formation (Asim et al., 2020). In plants, nitrate is sensed and transported by nitrate transporters NRT1.1 and NRT2.1. In addition to its role as an essential nutrient, nitrate also functions as a signal molecule that, within a vast signaling system, controls root system architecture (Asim et al., 2020). NRT gene expression is stimulated by N deficiency (Kiba et al., 2012), a condition which often arises in plants exposed to high salinity. In a previous study, we showed that in *Arabidopsis* seedlings, expression levels of *AtNRT1.1* and *AtNRT2.1* were induced by 50 mM NaCl, but priming with quinoa seed hull powder, which mitigated the negative effects of salinity, reverted this response (Ruiz et al., 2025). Present results showed that, at the higher NaCl concentration used here (100 mM), *NRT2.1* transcription was unaffected by salt, perhaps indicating an inability to activate this tolerance mechanism under strongly stressful conditions. Nevertheless, *AtNRT2.1* was upregulated by co-culture with A7 on SM (Fig. 4C). Higher expression of the nitrate transporter and the N-fixing ability of A7 could determine increased nitrate concentrations. The effect of nitrate on LR development is complex. However, expression of the auxin response factor ARF8 was shown to be strongly induced in pericycle cells in response to nitrate, thus promoting LR formation (Gifford et al., 2008). Conversely, we showed that *AtNRT1.1* was downregulated by A7 (Fig. 4D). Krouk et al. (2010) showed that in *Arabidopsis* NRT1.1 is also involved in auxin transport/efflux. According to these authors, NRT1.1 represses LR growth at low nitrate concentrations by promoting auxin transport out of these roots. Conversely, higher local nitrate (as in MS medium) would promote LR development via downregulation of NRT1.1-mediated auxin efflux (Lavenus et al., 2013). Exogenously supplied jasmonates (JAs) promote LR formation due to an increase in auxin biosynthesis in the root (Sun et al., 2009). *ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1* (*ASA1*) is a JA-regulated auxin biosynthesis gene. Results showed that its expression level decreased under saline conditions (Fig. 4E), in accord with a decrease in the number of LRs. On NSM, A7 enhanced the number of LRs and this correlated with upregulation of this gene (Fig. 4E). Instead, the increased number of LRs induced by A7 on SM was not accompanied by increased expression of *AtASA1*. The gene encoding for the auxin receptor Transport Inhibitor Response 1 (*AtTIR1)* was not affected by salt and was downregulated by A7 both on SM and NSM (Fig. 4F). Although available information is often contradictory, most abiotic and biotic stresses suppress the expression of *TIR1/AFB* family members (Du et al., 2022).

Synthesis of 5-aminolevulinic acid (ALA) is the rate-limiting step for the formation of all plant tetrapyrroles, including chlorophyll; thus, regulating ALA biosynthesis is critical for photosynthesis and, consequently, plant growth and development. Glutamyl-tRNA reductase (GluTR), the first specific enzyme of this pathway, is encoded by a family of nuclear *HEMA* genes. In antisense transgenic lines of *Arabidopsis*,

chlorophyll levels were proportional to the level of Glu-TR expression and were inversely related to the levels of antisense HEMA transcripts (Kumar and Söll, 2000). Results (Fig. 5A) showed that *AtHEMA* transcription was downregulated by salt and by A7 in the absence of salt; instead, on SM it was upregulated by A7 and thus coherent with the return to control values of Chl*a* and enhanced Chl*b* contents, thereby confirming its stress-mitigating capacity.

Glutaredoxins are small oxidoreductase proteins that reduce disulfide, and cysteine (Cys)-glutathione bonds in target proteins. Because Cys residues can be oxidized by reactive oxygen species, glutaredoxins act as antioxidants that reduce oxidative damage to proteins (Patterson et al., 2016). Several studies on the salt stress-mitigating effects of PGPR have shown that the enzymatic and non-enzymatic antioxidant machinery of the host plant was positively affected (Lastochkina et al., 2017; El-Esawi et al., 2019; Shabaan et al., 2022). Herein, we show that transcript levels of some members of this gene family (*AtGRX3* and ***4***) were downregulated by salt and that A7 restored them to control levels (Fig. 5B, C), thus corroborating its stress-mitigating effect via improving the plant's antioxidant status.

NHX and *SOS* are among the key genes affecting tissue tolerance under salinity conditions. Their expression often exhibits significant correlation with salt tolerance (Ruiz et al., 2011). Plant NHX transporters are Na^+/H^+ antiporters playing pivotal roles in the homeostasis of Na^+ and K^+ under salinity and drought stress (Santhoshi et al., 2025). The Salt Overly Sensitive (SOS) pathway is a major signaling pathway comprising various elements that ultimately control the activity of SOS1, a plasma membrane Na^+/H^+ exchanger governing efflux of Na^+ from roots, and Na^+ loading into xylem vessels for long-distance translocation to the leaves (Ali et al., 2023). In the present study, *AtNHX1* and *AtSOS1* were downregulated on SM (Fig. 6A, B), possibly suggesting that, under our conditions, this highly salt-sensitive plant was unable to activate these specific tolerance mechanisms based on ion exclusion/sequestration. In a comparative study on quinoa genotypes with different levels of salt tolerance, Ruiz et al. (2011) showed that *CqSOS1* was strongly induced in a genotype displaying a fairly high level of salt tolerance, whereas the more salt-sensitive genotype showed no alteration in the transcript levels of this gene; similarly, a significant increase in *CqNHX1* expression on SM occurred in shoots of all genotypes analysed, except in the most salt-sensitive one. On SM, however, co-culture with A7 restored *SOS1* transcript abundance to control levels (Fig. 6B), corroborating its capacity to mitigate salt stress. A study in *Arabidopsis* revealed an inverse correlation between *AtSOS1* expression and plant Na^+ content, (Jha et al., 2010). Thus, up-regulation of this gene in seedlings inoculated with A7 suggests that tolerance might be associated with enhanced efflux/reduced translocation of Na^+. The expression pattern of *AtHKT1* revealed a context-dependent regulation influenced by both salinity and bacterial inoculation (Fig. 6C). In plants exposed to NaCl, *AtHKT1* was significantly upregulated, consistent with its role in retrieving Na^+ from the xylem and limiting its translocation to the shoot, thereby maintaining ionic balance under salinity (Hamamoto et al., 2015). Conversely, inoculation with *Pseudomonas* strain A7 on NSM led to its downregulation, which aligns with the transporter's Na^+ specificity and lack of direct involvement in K^+ transport in dicots. Although in the absence of excess Na^+, HKT1 activity is physiologically unnecessary, its repression in A7-inoculated seedlings may reflect bacterial optimization of ion homeostasis. Beneficial *Pseudomonas* strains modulate root ion transport and hormonal signaling, particularly through IAA, ethylene, and ABA pathways, reducing the plant's constitutive need for stress-related transporters (Zhang et al., 2022). Under combined NaCl and A7 treatment, *AtHKT1* expression remained stable relative to NaCl alone, suggesting that bacterial inoculation attenuates salt stress intensity and stabilizes ion fluxes.

Proline is an osmolyte that accumulates in plants under salt and drought stress; it also has other important protective functions (Kaur and Asthir, 2015). *P5CS* encodes the key enzyme in the biosynthesis of proline, and its expression levels are generally upregulated under stress conditions. Consistent with its role in salt tolerance, *AtP5CS* was upregulated on SM in *Arabidopsis* seedlings (Fig. 6D) and this upregulation was not affected by the presence of A7. In its catabolic pathway, proline is converted to glutamate by two reactions, catalysed by proline dehydrogenase (ProDH) and Δ^1-pyrroline-5-carboxylate dehydrogenase (P5CDH), respectively. ProDH is developmentally regulated but also modulated by environmental stimuli. Drought, extreme temperatures, and salinity repress ProDH activity and stimulate proline synthesis, thus leading to net accumulation of the amino acid (Cecchini et al., 2011). Our results show that under saline conditions, *AtProDH1* and *2* were downregulated relative to the control both in the absence and presence of A7 (Fig. 6E, F), suggesting that proline catabolism was repressed so that, together with enhanced biosynthesis (*AtP5C5* upregulation), proline accumulation occurred. Although studies have shown that PGPB-induced salt tolerance can be accompanied by increased levels of proline in host plants, i.e., above those observed under salt treatment alone (Gupta et al., 2022; Khan et al., 2023; Mahgoub et al., 2021), we observed unchanged levels in inoculated *vs*. non inoculated plants, suggesting that salt stress alleviation by A7 was not dependent on this factor Interestingly, although A7 did not modify the salt-induced response of *Arabidopsis* seedlings, it enhanced proline above control levels in the absence of NaCl, as confirmed by the gene expression results for *AtP5CS* and *AtProDH*. This seed endophyte may, in fact, have evolved to prime quinoa seeds, for example by triggering proline accumulation, thereby preparing seedlings to cope with future exposure to high salinity. Similar results were obtained by Khan et al. (2023) who reported that *Brassica juncea* plants inoculated with two strains of PGPR displayed increased accumulation of proline and glycine betaine both under control and salt stress conditions.

5. Conclusions

In this study, the culturable fraction of the quinoa seed microbiota was characterized. Fifteen bacterial strains were isolated, assessed for their salt tolerance and physiological activities related to plant growth promotion, and subsequently identified. Interestingly, a predominance of halophilic *Staphylococcus* spp., belonging to the species *S. xylosus* and *S. aureus*, was observed. Among the isolated bacterial strains, *Pseudomonas putida* A7 exhibited the largest repertoire of plant-beneficial traits and was selected for further experiments.

Present results indicate that in the highly salt-sensitive model plant *Arabidopsis thaliana*, the negative impact of salinity on growth, and chlorophyll levels was mitigated by inoculation with strain A7. This salt stress-mitigating potential correlated with enhanced expression of several genes in inoculated *vs* non-inoculated plants. Further research on these genes could provide a mechanistic explanation of salt stress mitigation as a result of inoculation. We also showed that A7 modified seedling root architecture by enhancing lateral root formation, both under saline and non-saline conditions. This was presumably the result of its PGP activities, such as IAA and ACC deaminase production.

Future research could involve checking whether the microbiota of highly salt-tolerant quinoa ecotypes/varieties (such as Pandela) differs from that of less tolerant ones and to what extent this relates to soil characteristics (e.g. salinity). If so, this would corroborate the important role played by beneficial microbes on quinoa's stress tolerance. Undertaking such large-scale studies would be facilitated by using seeds, rather than the rhizosphere, as the source of bacteria. In fact, seed endophytes are: (i) vertically transmitted from one plant generation to the next, ensuring early and efficient colonization; (ii) more uniform since not directly affected by soil properties and less sensitive to environmental fluctuations typically occurring in the rhizosphere, (iii) the "starter microbiota" of the plant, potentially shaping the microbial community structure of later developmental stages.

Finally, the potential of quinoa-associated PGPB, whether culturable or non-culturable, acting as single strains or in consortia, to enhance stress resilience in salt-sensitive crops also under soil conditions (field or

pot experiments) represents a promising research direction, particularly in the context of climate change. Future studies should also address the characterization of the entire seed microbiota, which includes not only bacteria but also viruses, archaea, fungi, and protozoa, all together playing crucial roles in regulating plant growth and health.

CRediT authorship contribution statement

Karina B. Ruiz: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Investigation, Funding acquisition, Formal analysis, Data curation. **Giorgia Novello:** Writing – original draft, Investigation. **Ricardo Tejos:** Writing – review & editing, Validation, Resources, Investigation, Funding acquisition, Data curation, Conceptualization. **Sebastian Segovia-Ulloa:** Investigation. **Sebastián Sepúlveda-Villegas:** Visualization, Investigation, Formal analysis. **Matías Araya-Araya:** Investigation. **Gabriela Aguirre-Martínez:** Resources, Funding acquisition. **Fabiana Antognoni:** Writing – review & editing, Data curation. **Stefania Biondi:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Patrizia Cesaro:** Investigation. **Elisa Bona:** Validation, Investigation, Data curation. **Guido Lingua:** Writing – review & editing, Conceptualization. **Elisa Gamalero:** Writing – review & editing, Writing – original draft, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by A.N.I.D. INI_11190834, SINCPROYEXT-0325 PROFUNDIZA-UNAP (Chile) to K.B.R., FONDECYT Regular 1171442 (Chile) to R.T., SINCPROYEXT-0105 CONSOLIDA-UNAP (Chile) to G.A., and Ricerca Fondamentale Orientata (RFO) grants from the University of Bologna (Italy) to F.A. Francis Aceituno, technician in the Molecular Pharmacology laboratory at the Arturo Prat University, is acknowledged for her assistance in physiological and chemical analyses.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2026.101371.

Data availability

Data will be made available on request.

References

Agarwal, M., Sheikh, M.B., 2025. Isolation and functional characterization of endophytic bacteria from muscadine grape berries: a microbial treasure trove. Cells 14 (369). https://doi.org/10.3390/cells14050369.

Ali, A., Petrov, V., Yun, D.J., Gechev, T., 2023. Revisiting plant salt tolerance: novel components of the SOS pathway. Trends Plant Sci. 28, 1060–1069. https://doi.org/10.1016/j.tplants.2023.04.003.

Ali, B., Hafeez, A., Javed, M.A., Afridi, M.S., Abbasi, H.A., Qayyum, A., Batool, T., Ullah, A., Marc, R.A., Al Jaouni, S.K., Alkhalifah, D.H.M., Selim, S., 2022. Role of endophytic bacteria in salinity stress amelioration by physiological and molecular mechanisms of defense: a comprehensive review. South Afr. J. Bot. 151, 33–46. https://doi.org/10.1016/j.sajb.2022.09.036.

Alibrandi, P., Cardinale, M., Rahman, M.M., Strati, F., Ciná, P., de Viana, M.L., Giamminola, E.M., Gallo, G., Schnell, S., De Filippo, C., Ciaccio, M., Puglia, A.M., 2018. The seed endosphere of *Anadenanthera colubrina* is inhabited by a complex microbiota, including *Methylobacterium* spp. and *Staphylococcus* spp. with potential plant-growth promoting activities. Plant Soil 422, 81–99. https://doi.org/10.1007/s11104-017-3182-4.

Alsharif, W., Saad, M.M., Hirt, H., 2020. Desert microbes for boosting sustainable agriculture in extreme environments. Front. Microbiol. 11, 1666. https://doi.org/10.3389/fmicb.2020.01666.

Altamura, M.M, Piacentini, D., Della Rovere, F., Fattorini, L., Falasca, G., Betti, C., 2023. New paradigms in brassinosteroids, strigolactones, sphingolipids, and nitric oxide interaction in the control of lateral and adventitious root formation. Plants 12, 413. https://doi.org/10.3390/plants12020413.

Ashraf, M., Shahida, H., Odile, B., Tariq, M., 2004. Inoculating wheat seedling with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol. Fertil. Soils. 40, 157–162. https://doi.org/10.1007/s00374-004-0766-y.

Asim, M., Ullah, Z., Xu, F., An, L., Aluko, O.O., Wang, Q., Liu, H., 2020. Nitrate signaling, functions, and regulation of root system architecture: insights from *Arabidopsis thaliana*. Genes 11, 633. https://doi.org/10.3390/genes11060633.

Ayaz, M., Ali, Q., Jiang, Q., Wang, R., Wang, Z., Mu, G., Khan, S.A., Khan, A.R., Manghwar, H., Wu, H., Gao, X., 2022. Salt tolerant *Bacillus* strains improve plant growth traits and regulation of phytohormones in wheat under salinity stress. Plants 11, 2769. https://doi.org/10.3390/plants11202769.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline in water-stress studies. Plant Soil 39, 205–207.

Bhagat, N., Raghav, M., Dubey, S., Bedi, N., 2021. Bacterial exopolysaccharides: insight into their role in plant abiotic stress tolerance. J. Microbiol. Biotechnol. 31, 1045–1059. https://doi.org/10.4014/jmb.2105.05009.

Cecchini, N.M, Monteoliva, M.I., Alvarez, M.E., 2011. Proline dehydrogenase contributes to pathogen defense in *Arabidopsis*. Plant Physiol. 155, 1947–1959. https://doi.org/10.1104/pp.110.167163.

Chu, T.N., Tran, B.T.H., Van Bui, L., Hoang, M.T.T., 2019. Plant growth-promoting rhizobacterium *Pseudomonas* PS01 induces salt tolerance in *Arabidopsis thaliana*. BMC Res. Notes 12, 1–7. https://doi.org/10.1186/s13104-019-4046-1.

Cordova-Rodriguez, A., Rentería-Martínez, M.E., López-Miranda, C.A., Guzmán-Ortíz, J. M., Moreno-Salazar, S.F., 2022. Simple and sensitive spectrophotometric method for estimating the nitrogen-fixing capacity of bacterial cultures. MethodsX 9, 101917. https://doi.org/10.1016/j.mex.2022.101917.

Dimkpa, C., Weinand, T., Asch, F., 2009. Plant–rhizobacteria interactions alleviate abiotic stress conditions. Plant, Cell Environ. 32, 1682–1694. https://doi.org/10.1111/j.1365- 3040.2009.02028.x.

Du, W., Lu, Y., Li, Q., Luo, S., Shen, S., Li, N., Chen, X., 2022. TIR1/AFB proteins: active players in abiotic and biotic stress signaling. Front. Plant Sci. 13, 1083409. https://doi.org/10.3389/fpls.2022.1083409.

Dworkin, M., Foster, J., 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol. 75, 592–603.

Egamberdieva, D., Jabborova, D., Hashem, A., 2015. *Pseudomonas* induces salinity tolerance in cotton (*Gossypium hirsutum*) and resistance to *Fusarium* root rot through the modulation of indole-3-acetic acid. Saudi J. Biol. Sci. 22, 773–779. https://doi.org/10.1016/j.sjbs.2015.04.019.

Eida, A.A., Ziegler, M., Lafi, F.F., Michell, C.T., Voolstra, C.R., Hirt, H., Saad, M.M., 2018. Desert plant bacteria reveal host influence and beneficial plant growth properties. PLoS One 13, e0208223. https://doi.org/10.1371/journal.pone.0208223.

El-Esawi, M.A., AA, Al-Ghamdi, Ali, H.M., Alayafi, A.A., 2019. *Azospirillum lipoferum* FK1 confers improved salt tolerance in chickpea (*Cicer arietinum* L.) by modulating osmolytes, antioxidant machinery and stress-related genes expression. Environ. Exp. Bot. 159, 55–65. https://doi.org/10.1016/j.envexpbot.2018.12.001.

Fagorzi, C., Mengoni, A., 2022. Endophytes: improving plant performance. Microorganisms 10, 1777. https://doi.org/10.3390/microorganisms10091777.

Gamalero, E., Favale, N., Bona, E., Novello, G., Cesaro, P., Massa, N., Glick, B.R., Orozco-Mosqueda, M.C., Berta, G., Lingua, G., 2020. Screening of bacterial endophytes able to promote plant growth and increase salinity tolerance. Appl. Sci. 10, 5767. https://doi.org/10.3390/app10175767.

Gamalero, E., Glick, B., 2011. Mechanisms used by plant growth-promoting bacteria. In: Maheshwari, D.K. (Ed.), Bacteria in Agrobiology: Plant Nutrient Management. Springer-Verlag, Berlin Heidelberg, pp. 17–46. https://doi.org/10.1007/978-3-642-21061-7_2.

Gifford, M.L., Dean, A., Gutierrez, R.A., Coruzzi, G.M., Birnbaum, K.D., 2008. Cell-specific nitrogen responses mediate developmental plasticity. Proc. Natl. Acad. Sci. U.S.A. 105, 803–808. https://doi.org/10.1073/pnas.0709559105.

Glick, B.R., Cheng, Z., Czarny, J., Duan, J., 2007. Promotion of plant growth by ACC deaminase-containing soil bacteria. Eur. J. Plant Pathol. 119, 329–339. https://doi.org/10.1007/s10658-007-9162-4.

Gonin, M., Salas-González, I., Gopaulchan, D., Frene, J.P., Roden, S., Van de Poel, B., Salt, D.E., Castrillo, G., 2023. Plant microbiota controls an alternative root branching regulatory mechanism in plants. Proc. Natl. Acad. Sci. U.S.A. 120, e2301054120. https://doi.org/10.1073/pnas.2301054120.

González-Teuber, M., Urzúa, A., Plaza, P., Bascuñán-Godoy, L., 2018. Effects of root endophytic fungi on response of *Chenopodium quinoa* to drought stress. Plant Ecol. 219, 231–240. https://doi.org/10.1007/S125-8-017-079 1-1.

Gupta, A., Rai, S., Bano, A., Sharma, S., Kumar, M., Binsuwaidan, R., Suhail Khan, M., Upadhyay, T.K., Alshammari, N., Saeed, M., et al., 2022. ACC deaminase produced by PGPR mitigates the adverse effect of osmotic and salinity stresses in *Pisum sativum* through modulating the antioxidants activities. Plants 11, 3419. https://doi.org/10.3390/plants11243419.

Gupta, S., Pandey, S., 2019. ACC deaminase-producing bacteria with multifarious plant growth-promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants. Front. Microbiol. 10, 1506. https://doi.org/10.3389/fmicb.2019.01506.

Hamamoto, S., Horie, T., Hauser, F., Deinlein, U., Schroeder, J.I., Uozumi, N., 2015. HKT transporters mediate salt stress resistance in plants: from structure and function to

the field. Curr. Op. Biotechnol. 32, 113–120. https://doi.org/10.1016/j.copbio.2014.11.025.
Hardoim, P.R., Hardoim, C.C., Van Overbeek, L.S., Van Elsas, J.D., 2012. Dynamics of seed-borne rice endophytes on early plant growth stages. PLoS One 7, e30438. https://doi.org/10.1371/journal.pone.0030438.
Hernandez, I., Taulé, C., Pérez-Pérez, R., Battistoni, F., Fabiano, E., Villanueva-Guerrero, A., Nápoles, M.C., Herrera, H., 2023. Endophytic seed-associated bacteria as plant growth promoters of Cuban rice (*Oryza sativa* L.). Microorganisms 11, 2317. https://doi.org/10.3390/microorganisms11092317.
Hu, X., Chen, J., Guo, J., 2006. Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World J. Microb. Biotechnol. 22, 983–990. https://doi.org/10.1007/s11274-006-9144-2.
Jacobsen, S.E., Mujica, A., Jensen, C.R., 2003. The resistance of quinoa (*Chenopodium quinoa* Willd.) to adverse abiotic factors. Food Rev. Int. 19, 99–109.
Jalili, F., Khavazi, K., Pazira, E., Nejati, A., Rahmani, H.A., Sadaghiani, H.R., et al., 2009. Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate salinity stress on canola (*Brassica napus* L.) growth. J. Plant Physiol. 166, 667–674. https://doi.org/10.1016/j.jplph.2008.08.004.
Javid, M.G., Sorooshzadeh, A., Moradi, F., Sanavy, S.A.M.M., Allahdadi, I., 2011. The role of phytohormones in alleviating salt stress in crop plants. Aust. J. Crop. Sci. 5, 726–734.
Jha, D., Shirley, N., Tester, M., Roy, S.J., 2010. Variation in salinity tolerance and shoot sodium accumulation in *Arabidopsis* ecotypes linked to differences in the natural expression levels of transporters involved in sodium transport. Plant Cell Environ. 33, 793–804. https://doi.org/10.1111/j.1365-3040.2009.02105.x.
Kaur, G., Asthir, B., 2015. Proline: a key player in plant abiotic stress tolerance. Biol. Plant. 59, 609–619. https://doi.org/10.1007/s10535-015-0549-3.
Khan, M.A., Sahile, A.A., Jan, R., Asaf, S., Hamayun, M., Imran, M., et al., 2021. Halotolerant bacteria mitigate the effects of salinity stress on soybean growth by regulating secondary metabolites and molecular responses. BMC Plant Biol. 21, 176. https://doi.org/10.1186/s12870-021-02937-3.
Khan, V., Umar, S., Iqbal, N., 2023. Palliating salt stress in mustard through plant growth-promoting rhizobacteria: regulation of secondary metabolites, osmolytes, antioxidative enzymes and stress ethylene. Plants 12, 705. https://doi.org/10.3390/plants12040705.
Khoso, M.A., Wagan, S., Alam, I., Hussain, A., Ali, Q., Saha, S., Poudel, T.R., Manghwar, H., Liu, F., 2024. Impact of plant growth-promoting rhizobacteria (PGPR) on plant nutrition and root characteristics: current perspective. Plant Stress 11, 100341. https://doi.org/10.1016/j.stress.2023.100341.
Kiba, T., Feria-Bourrellier, A.B., Lafouge, F., Lezhneva, L., Boutet-Mercey, S., Orsel, M., Bréhaut, V., Miller, A., Daniel-Vedele, F., Sakakibara Krapp, A., 2012. The *Arabidopsis* nitrate transporter NRT2.4 plays a double role in roots and shoots of nitrogen-starved plants. Plant Cell 24, 245–258. https://doi.org/10.1105/tpc.111.092221.
Kim, J., Ahn, S.H., Yang, J.S., Choi, S., Jung, H.W., Jeon, J., 2023. Plant protective and growth promoting effects of seed endophytes in soybean plants. Plant Pathol. J 39, 513–521. https://doi.org/10.5423/PPJ.OA.06.2023.0091.
Krouk, G., Lacombe, B., Bielach, A., Perrine-Walker, F., Malinska, K., Mounier, E., Hoyerova, K., Tillard, P., Leon, S., Ljung, K., Zazimalova, E., Benkova, E., Nacry, P., Gojon, A., 2010. Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. Dev. Cell 18, 927–937. https://doi.org/10.1016/j.devcel.2010.05.008.
Kumar, A., Kumar Solanki, M., Wang, Z., Chandrol Solanki, A., Kumar Singh, V., Kumar Divvela, P., 2024. Revealing the seed microbiome: navigating sequencing tools, microbial assembly, and functions to amplify plant fitness. Microbiol. Res. 279, 127549. https://doi.org/10.1016/j.micres.2023.127549.
Kumar, A.M., Söll, D., 2000. Antisense *HEMA1* RNA expression inhibits heme and chlorophyll biosynthesis in *Arabidopsis*. Plant Physiol. 122, 49–56. https://doi.org/10.1104/pp.122.1.49.
Kumar, M., Prasad, V., 2025. Growth and functional evaluation of *Enterobacter cloacae* under salinity stress. Biocatal. Agric. Biotechnol. 64, 103495. https://doi.org/10.1016/j.bcab.2025.103495.
Kumar, P., Dubey, R., Maheshwari, D., 2012. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol. Res. 167, 493–499. https://doi.org/10.1016/j.micres.2012.05.002.
Kumar, S., Anwer, R., Yadav, M., Sehrawat, N., Singh, M., Kumar, V., 2021. Molecular typing and global epidemiology of *Staphylococcus aureus*. Curr. Pharmacol. Rep. 7, 179–186. https://doi.org/10.1007/s40495-021-00264-7.
Lastochkina, O., Pusenkova, L., Yuldashev, R., Babaev, M., Garipova, S., Blagova, D., Khairullin, R., Aliniaeifard, S., 2017. Effects of *Bacillus subtilis* on some physiological and biochemical parameters of *Triticum aestivum* L.(wheat) under salinity. Plant Physiol. Biochem. 121, 80–88. https://doi.org/10.1016/j.plaphy.2017.10.020.
Lavenus, J., Goh, T., Roberts, I., Guyomarc'h, S., Lucas, M., De Smet, I., Fukaki, H., et al., 2013. Lateral root development in *Arabidopsis*: fifty shades of auxin. TIPS 18, 450–458. https://doi.org/10.1016/j.tplants.2013.04.006.
Lebrazi, S., Niehaus, K., Bednarz, H., Fadil, M., Chraibi, M., Benbrahim, K.F., 2020. Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. J. Genet. Eng. Biotechnol. 18, 71. https://doi.org/10.1186/s43141-020-00090-2.
Lewis, D.R., Negi, S., Sukumar, P., Muday, G.K., 2011. Ethylene inhibits lateral root development, increases IAA transport and expression of *PIN3* and *PIN7* auxin efflux carriers. Development 138, 3485–3495. https://doi.org/10.1242/dev.065102.
Li, Z., Jiang, J., Sun, K., Ye, S., 2025. Germination of peanut seeds promoted by an endophytic *Priestia megaterium* PH3 via activating ROS and hormone metabolism pathway under salt stress. Plant, Cell Environ. 48, 7426–7439. https://doi.org/10.1111/pce.70040.
Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382.
Lin, J.-H., Yu, L.-H., Xiang, C.-B, 2020. Arabidopsis nitrate regulated1 acts as a negative modulator of seed germination by activating *ABI3* expression. New Phytol. 225, 835–847. https://doi.org/10.1111/nph.16172.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using realtime quantitative PCR and the $2(-^{\Delta\Delta CT})$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.
Louden, B.C., Haarmann, D., Lynne, A.M., 2011. Use of blue agar CAS assay for siderophore detection. J. Microbiol. Biol. Educ. 12, 51–53. https://doi.org/10.1128/jmbe.v12i1.249.
Luzio, L.W., 2010. Suelos de Chile. Chapter 2. Impreso Maval, Santiago, Chile, pp. 39–57. ISBN N° 978-956-19-0648-8.
Maestro-Gaitán, I., Granado-Rodríguez, S., Redondo-Nieto, M., Battaglia, A., Poza-Viejo, L., Matías, J., et al., 2023. Unveiling changes in rhizosphere-associated bacteria linked to the genotype and water stress in quinoa. Microb. Biotechnol. 16, 2326–2344. https://doi.org/10.1111/1751-7915.14337.
Maestro-Gaitán, I., Redondo-Nieto, M., González-Bodí, S., Rodríguez-Casillas, L., Matías, J., Bolaños, L., Reguera, M., 2025. Insights into quinoa endophytes: core bacterial communities reveal high stability to water stress and genotypic variation. Environ. Microbiome 20, 16. https://doi.org/10.1186/s40793-025-00673-x.
Mahgoub, H.A.M., Fouda, A., Eid, A.M, El-Din Ewais, E., El-Din Hassan, S., 2021. Biotechnological application of plant growth-promoting endophytic bacteria isolated from halophytic plants to ameliorate salinity tolerance of *Vicia faba* L. Plant Biotechnol. Rep. 15, 819–843. https://doi.org/10.1007/s11816-021-00716-y.
Mastretta, C., Taghavi, S., van der Lelie, D., Mengoni, A., Galardi, F., Gonnelli, C., 2009. Endophytic bacteria from seeds of *Nicotiana tabacum* can reduce cadmium phytotoxicity. Int. J. Phytoremed. 11, 251–267. https://doi.org/10.1080/15226510802432678.
Melini, V., Melini, F., 2021. Functional components and anti-nutritional factors in gluten-free grains: a focus on quinoa seeds. Foods 10, 351. https://doi.org/10.3390/foods10020351.
Miño, R., Ballesteros, G.I., Ruiz, K.B., Acuña-Rodríguez, I.S., Molina-Montenegro, M.A., 2025. Fungal endophytes boost salt tolerance and seed quality in quinoa ecotypes along a latitudinal gradient. Front. Plant Sci. 16, 1602553. https://doi.org/10.3389/fpls.2025.1602553.
Murashige, T., Skoog, F.A., 1962. revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.
Novello, G., Bona, E., Toumatia, O., Vuolo, F., Bouras, N., Titouah, H., Zitouni, A., Gorrasi, S., Massa, N., Cesaro, P., Todeschin, V., Lingua, G., Gamalero, E., 2023. Rhizosphere bacterial isolation from indigenous plants in arid and semi-arid Algerian soils: implications for plant growth enhancement. Processes 11, 2907. https://doi.org/10.3390/pr11102907.
Orozco-Mosqueda, M.C., Glick, B.R., Santoyo, G., 2020. ACC deaminase in plant growth-promoting bacteria (PGPB): an efficient mechanism to counter salt stress in crops. Microb. Res. 235, 126439. https://doi.org/10.1016/j.micres.2020.126439.
Pal, K.K., Dey, R., Sherathia, D.N., Devidayal, Mangalassery S., Kumar, A., Rupapara, R. B., Mandaliya, M., et al., 2021. Alleviation of salinity stress in peanut by application of endophytic bacteria. Front. Microbiol. 12, 650771. https://doi.org/10.3389/fmicb.2021.650771.
Parfentjev, I., Catelli, A.R., 1964. Tolerance of *Staphylococcus aureus* to sodium chloride. J. Bacteriol. 88, 1–3. https://doi.org/10.1128/jb.88.1.1-3.1964.
Patterson, K., Walters, L.A., Cooper, A.M., Olvera, J.G., Rosas, M.A., Rasmusson, A.G., Escobar, M.A., 2016. Nitrate-regulated glutaredoxins control Arabidopsis primary root growth. Plant Physiol. 170, 989–999. https://doi.org/10.1104/pp.15.01776.
Payne, S.M., 1994. Detection, isolation, and characterization of siderophores. Methods Enzymol. 235, 329–344. https://doi.org/10.1016/0076-6879(94)35151-1.
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.
Pérez-Torres, C.A., López-Bucio, J., Cruz-Ramírez, A., Ibarra-Laclette, E., Dharmasiri, S., Estelle, M., Herrera-Estrella, L., 2008. Phosphate availability alters lateral root development in Arabidopsis by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. Plant Cell 20, 3258–3272. https://doi.org/10.1105/tpc.108.058719.
Pitzschke, A., 2016. Developmental peculiarities and seed-borne endophytes in quinoa: omnipresent, robust bacilli contribute to plant fitness. Front. Microbiol. 7, 2. https://doi.org/10.3389/fmicb.2016.00002.
Prado, F.E., Fernández-Turiel, J.L., Tsarouchi, M., Psaras, G.K., González, J.A., 2014. Variation of seed mineral concentrations in seven quinoa cultivars grown in two agroecological sites. Cereal Chem. 91, 453–459. https://doi.org/10.1094/CCHEM-08-13-0157-R.
Puente, M.E., Li, C.Y., Bashan, Y., 2009. Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. Environ. Exp. Bot. 66, 402–408. https://doi.org/10.1016/j.envexpbot.2009.04.007.
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.
Ragel, P., Raddatz, N., Leidi, E.O., Quintero, F.J., Pardo, J.M., 2019. Regulation of K^+ nutrition in plants. Front. Plant Sci. 10, 281. https://doi.org/10.3389/fpls.2019.00281.

Romão, I.R., do, Carmo, Gomes, J., Silva, D., Vilchez, J.I., 2025. The seed microbiota from an application perspective: an underexplored frontier in plant–microbe interactions. Crop Health 3, 12. https://doi.org/10.1007/s44297-025-00051-6.

Ruiz, K.B., Antognoni, F., Konotie Coulibaly, A., Lizardi, S., Covarrubias, A., Martínez, E. A., Molina-Montenegro, M.A., Biondi, S., Zurita-Silva, A., 2011. Variation in salinity tolerance of four lowland genotypes of quinoa (*Chenopodium quinoa* Willd.) as assessed by growth, physiological traits, and sodium transporter gene expression. Plant Physiol. Biochem. 49, 1333–1341. https://doi.org/10.1016/j.plaphy.2011.08.005.

Ruiz, K.B., Lianza, M., Segovia-Ulloa, S., Sepúlveda-Villegas, S., Salas, F., Tejos, R., Biondi, S., Antognoni, F., 2025. Priming with quinoa dehulling residues induces changes in gene expression, boosts antioxidant defense, and mitigates salt stress in *Arabidopsis thaliana* L. Plant Physiol. Biochem. 222, 109704. https://doi.org/10.1016/j.plaphy.2025.109704.

Ruiz, K.B., Maldonado, J., Biondi, S., Silva, H., 2019. RNA-seq analysis of salt-stressed *versus* non salt-stressed transcriptomes of *Chenopodium quinoa* landrace R49. Genes 10, 1042. https://doi.org/10.3390/genes10121042.

Ruiz, K.B., Rapparini, F., Bertazza, G., Silva, H., Torrigiani, P., Biondi, S., 2017. Comparing salt-induced responses at the transcript level in a salares and coastal-lowlands landrace of quinoa (*Chenopodium quinoa* Willd). Environ. Exp. Bot. 139, 127–142. https://doi.org/10.1016/j.envexpbot.2017.05.003.

Santhoshi, Y., Anjana, A.B., Zala, H., Bosamia, T., Tiwari, K., Prajapati, K., Patel, P., Soni, N., Patel, N., Solanki, S., et al., 2025. Comprehensive analysis of the NHX gene family and its regulation under salt and drought stress in quinoa (*Chenopodium quinoa* Willd.). Genes 16, 70. https://doi.org/10.3390/genes16010070.

Sanz, L., Dewitte, W., Forzani, C., Patell, F., Nieuwland, J., Wen, B., Quelhas, P., De Jager, S., Titmus, C., Campilho, A., et al., 2011. The Arabidopsis D-type cyclin CYCD2;1 and the inhibitor ICK2/KRP2 modulate auxin-induced lateral root formation. Plant Cell 23, 641–660. https://doi.org/10.1105/tpc.110.080002.

Schmitz, L., Yan, Z., Schneijderberg, M., de Roij, M., Pijnenburg, R., Zheng, Q., Franken, C., et al., 2022. Synthetic bacterial community derived from a desert rhizosphere confers salt stress resilience to tomato in the presence of a soil microbiome. ISME J. 16, 1907–1920. https://doi.org/10.1038/s41396-022-01238-3.

Schwyn, B., Neilands, J., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47–56. https://doi.org/10.1016/0003-2697(87)90612-9.

Shabaan, M., Asghar, H.N., Zahir, Z.A., Zhang, X., Sardar, M.F., Li, H., 2022. Salt-tolerant PGPR confer salt tolerance to maize through enhanced soil biological health, enzymatic activities, nutrient uptake and antioxidant defense. Front. Microbiol. 13, 901865. https://doi.org/10.3389/fmicb.2022.901865.

Simon, P., 2003. Q-Gene: processing quantitative real-time RT–PCR data. Bioinformatics 19, 1439–1440. https://doi.org/10.1093/bioinformatics/btg157.

Singh, R.P., Kumari, K., 2023. Genome sequence of environmental isolate *Staphylococcus aureus* OS-6, isolated from a soil sample. Microbiol. Resour. Announc. 12, e0023823. https://doi.org/10.1128/mra.00238-23.

Singh, A.K., Sharma, R.K., Sharma, V., Singh, T., Kumar, R., Kumari, D., 2017. Isolation, morphological identification and in vitro antibacterial activity of endophytic bacteria isolated from *Azadirachta indica* (neem) leaves. Vet. World 10, 510–516. https://doi.org/10.14202/vetworld.2017.510-516.

Soussi, A., Ferjani, R., Marasco, R., Guesmi, A., Cherif, H., Rolli, E., Mapelli, F., Imene Ouzari, H., Daffonchio, D., Cherif, A., 2016. Plant-associated microbiomes in arid lands: diversity, ecology and biotechnological potential. Plant Soil 405, 357–370. https://doi.org/10.1007/s11104-015-2650-y.

Sun, J., Xu, Y., Ye, S., Jiang, H., Chen, Q., Liu, F., Zhou, W., Chen, R., Li, X., Tietz, O., Wu, X., Cohen, J.D., Palme, K., Li, C., 2009. *Arabidopsis* ASA1 is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. Plant Cell 21, 1495–1511. https://doi.org/10.1105/tpc.108.064303.

Taheri, P., Puopolo, G., Santoyo, G., 2025. Plant growth-promoting microorganisms: new insights and the way forward. Microb. Res. 297, 128168. https://doi.org/10.1016/j.micres.2025.128168.

Truyens, S., Weyens, N., Cuypers, A., Vangronsveld, J., 2015. Bacterial seed endophytes: genera, vertical transmission and interaction with plants. Environ. Microbiol. Rep. 7, 40–50. https://doi.org/10.1111/1758-2229.12181.

Venturi, V., Weisbeek, P., Koster, M., 1995. Gene regulation of siderophore-mediated iron acquisition in *Pseudomonas*: not only the Fur repressor. Mol. Microbiol. 17, 603–610. https://doi.org/10.1111/j.1365-958.1995.mmi 17040603.x.

Villota-Calvachi, G.E., González, K., Marulanda, S., Galeano, N., et al., 2022. Aislamiento y caracterización de bacterias productoras de biopolimeros a partir de efluentes industriales. Rev. Colomb. Biotecnol. 24, 27–45. https://doi.org/10.15446/rev.colomb.biote.v24n1.76660.

Wael, D., El-Amier, Y., Saber, W.I.A., Elsayed, A., 2024. Plant-associated halotolerant bacteria improving growth of *Vicia faba* L. Mariout-2 under salinity conditions. Sci. Rep. 14, 16737. https://doi.org/10.1038/s41598-024-66504-0.

Whatmore, A.M., Chudek, J.A., Reed, R.H., 1990. The effects of osmotic upshock on the intracellular solute pools of *Bacillus subtilis*. Microbiology 136, 2527–2535. https://doi.org/10.1099/00221287-136-12-2527.

Xu, M., Sheng, J., Chen, L., Men, Y., Gan, L., Guo, S., et al., 2014. Bacterial community compositions of tomato (*Lycopersicum esculentum* Mill.) seeds and plant growth promoting activity of ACC deaminase producing *Bacillus subtilis* (HYT-12-1) on tomato seedlings. World J. Microbiol. Biotechnol. 30, 835–845. https://doi.org/10.1007/s11274-013-1486-y.

Yang, A., Akhtar, S.S., Iqbal, S., Amjad, M., Naveed, M., Zahir, Z.A., Jacobsen, S.-E., 2016. Enhancing salt tolerance in quinoa by halotolerant bacterial inoculation. Funct. Plant Biol. 43, 632–642. https://doi.org/10.1071/FP15265.

Zahra, S.T., Tariq, M., Abdullah, M., Ullah, M.K., Rafiq, A.R., Siddique, A., Shahid, M.S., et al., 2024. Salt-tolerant plant growth-promoting bacteria (ST-PGPB): an effective strategy for sustainable food production. Curr. Microbiol. 81, 304. https://doi.org/10.1007/s00284-024-03830-6.

Zeng, F., Zheng, C., Ge, W., Gao, Y., Pan, X., Ye, X., Wu, X., Sun, Y., 2024. Regulatory function of the endogenous hormone in the germination process of quinoa seeds. Front. Plant Sci. 14, 1322986. https://doi.org/10.3389/fpls.2023.1322986.

Zeng, X., He, L., Guo, X., Deng, L., Yang, W., Zhu, Q., Duan, Z., 2017. Predominant processing adaptability of *Staphylococcus xylosus* strains isolated from Chinese traditional low-salt fermented whole fish. Int. J. Food Microbiol. 242, 141–151. https://doi.org/10.1016/j.ijfoodmicro.2016.11.014.

Zhang, H., Forde, B.G., 2000. Regulation of *Arabidopsis* root development by nitrate availability. J. Exp. Bot. 51, 51–59. https://doi.org/10.1093/jexbot/51.342.51.

Zhang, H., Kim, M.-S., Krishnamurthy, P., Park, S.-H., 2022. Microbe-mediated modulation of ion transporters enhances plant salt tolerance: molecular and physiological perspectives. Plant Physiol. Biochem. 183, 1–13. https://doi.org/10.1016/j.plaphy.2022.01.002.

Zhao, L., Wang, P., Hou, H., Zhang, H., Wang, Y., Yan, S., Huan, Y., L, H., Ta, J., H, A., Ga, F., 2014. Transcriptional regulation of cell cycle genes in response to abiotic stresses correlates with dynamic changes in histone modifications in maize. PLoS One 9, e106070. https://doi.org/10.1371/journal.pone.0106070.

Zhou, J., Li, P., Meng, D., Gu, Y., Zheng, Z., Yin, H., Zhou, Q., Li, J., 2020. Isolation, characterization and inoculation of Cd tolerant rice endophytes and their impacts on rice under Cd contaminated environment. Environ. Pollut. 260, 113990. https://doi.org/10.1016/j.envpol.2020.113990.

Zou, Y., Zhang, Y., Testerinket, C., 2021. Root dynamic growth strategies in response to salinity. Plant Cell Environ. 45, 695–704. https://doi.org/10.1111/pce.14205.