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RESEARCH ARTICLE

The halotolerant rizhobacterium *Glutamicibacter* sp. alleviates salt impact on *Phragmites australis* by producing exopolysaccharides and limiting plant sodium uptake

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Abstract

Salinity is a widespread abiotic stress, which has strong adverse effects on plant growth and crop productivity. Exopolysaccharides (EPS) play a crucial role in plant growth-promoting rhizobacteria (PGPR)-mediated improvement of plant stress tolerance. This study aimed to assess whether *Glutamicibacter* sp. strain producing large amounts of EPS may promote tolerance of common reed, *Phragmites australis* (Cav.) Trin. ex Steud., towards salt stress. This halotolerant rizhobacterium showed tolerance to salinity (up to 1 M NaCl) when cultivated on Luria-Bertani (LB) medium. Exposure to high salinity (300 mM NaCl) significantly impacted the plant growth parameters, but this adverse effect was mitigated following inoculation with *Glutamicibacter* sp., which triggered higher number of leaves and tillers, shoot fresh weight/dry weight, and root fresh weight as compared to non-inoculated plants. Salt stress increased the accumulation of malondialdehyde (MDA), polyphenols, total soluble sugars (TSSs), and free proline in shoots. In comparison, the inoculation with *Glutamicibacter* sp. further increased shoot polyphenol content, while decreasing MDA and free proline contents. Besides, this bacterial strain increased tissue Ca^{+} and K^{+} content concomitant to lower shoot Na^{+} and root Cl^{-} accumulation, thus further highlighting the beneficial effect of *Glutamicibacter* sp. strain on the plant behavior under salinity. As a whole, our study provides strong arguments for a potential utilization of EPS-producing bacteria as a useful microbial inoculant to alleviate the deleterious effects of salinity on plants.

KEYWORDS
exopolysaccharides, ion concentrations, osmolytes, PGPR, *Phragmites australis*, salinity stress

1 | INTRODUCTION

Various environmental stresses such as flood, extreme temperatures, soil/water salinization, and drought impact the growth and development of plants and ultimately cause substantial decline in crop yield (Bano & Fatima, 2009; Jha et al., 2011). Under the ongoing climate changes, soil salinity is an expanding environmental issue, affecting millions of hectares of land around the world and resulting in enormous economic losses each year (Munns & Gilliham, 2015). According to Munns (2005), salinity affects plants' growth and development through two main physiological pathways (osmotic stress and ion toxicity). The first phase appears immediately upon salt stress exposure and is due to

the osmotic stress caused by hypertonic conditions whereas the second phase occurs over several hours to days and weeks to develop and results from the toxic effects of salt ions (Na^+ and Cl^-) accumulating in the cells. Salt tolerance is known as a complex quantitative trait that is controlled by multiple genes and involves various physiological and biochemical mechanisms, which are also species specific (Chinnusamy et al., 2005; Flowers & Colmer, 2008; Shabala, 2013).

Common reed, *Phragmites australis* (Cav.) Trin. ex Steud., is a perennial reed grass considered as one of the most extensively distributed and productive fodder species in the world (Brix & Cizkova, 2001). *P. australis* is a species of major environmental significance due to its high intraspecific diversity, phenotypic plasticity, and evolutionary potential. *P. australis* also contributes to storm protection, sediment stabilization, and water filtration (Eller et al., 2017; Knight et al., 2018; Meyerson et al., 2009; Rodríguez & Brisson, 2015). In Tunisia, *P. australis* is frequent on coastal habitats, including shallow marshes, coastal mudflat areas, fringes of lagoons, and salt-affected wetlands (Gorai et al., 2010), and it is often the single dominant and keystone species in its habitats (Liu et al., 2018). High salinity levels restrict biomass production of *P. australis*, thereby leading to reduced establishment capacity and vigor in brackish and salt marshes (Eller et al., 2017). Pagter et al. (2009) hypothesized that this may be due to both osmotic and ion-specific effects. Therefore, it is essential to improve *P. australis* salt stress tolerance to minimize yield losses in a sustainable method.

Using salt-tolerant microorganisms to boost the growth of salt-stressed plants is a promising strategy to alleviate salinity (Dodd & Perez-Alfocea, 2012; Egamberdieva et al., 2019). Furthermore, the application of halotolerant microorganisms for salinity stress management opens interesting challenges for using pyramiding strategies against salinity, as well as new biological methods to elucidate further mechanisms of action in plant stress tolerance (Dodd & Perez-Alfocea, 2012; Kumar et al., 2018). Plant growth-promoting rhizobacteria (PGPR), which colonize the rhizosphere of various plants, can promote plant growth and alleviate salt stress without harming the environment. In the last decade, a large number of reports documented that bacteria of various genera including *Rhizobium*, *Bacillus*, *Pseudomonas*, *Azotobacter*, *Azospirillum*, and *Enterobacter* conferred better behavior to their host plants against different abiotic stresses such as salinity (Egamberdieva et al., 2019; Etesami & Beattie, 2017; Etesami & Glick, 2020; Kumar et al., 2020; Sarkar et al., 2018). This PGPR capacity is notably ascribed to (i) the improvement of plant nutrition by increasing the solubilization of phosphorus and the potassium availability, biological nitrogen fixation, and iron sequestration, (ii) bacteria secretion of metabolic products (1-aminocyclopropane-1-carboxylate [ACC] deaminase, which converts the plant ethylene substrate ACC to ammonia [NH_3] and alpha-ketobutyrate [$C_4H_6O_3$], plant growth-promoting hormones [mainly indole-3-acetic acid, IAA], exopolysaccharides [EPS], microorganism volatile organic compounds, and siderophores), and (iii) the activation of plant antioxidant defense mechanisms to alleviate oxidative damages in plants challenged with abiotic constraints (Bano & Fatima, 2009; Kohler et al., 2009; Kumar et al., 2018; Ullah et al., 2015).

High concentrations of Na^+ and Cl^- in the rhizosphere may inhibit nutrient-related activities and change ion balance as Na^+/Ca^{2+}, Na^+/K^+, Na^+/Mg^{2+}, Cl^-/NO_3^-, and Cl^-/H_2PO_4 ratios (Grattan & Grieve, 1998). Such phenomenon may cause an imbalance in the plant ionic composition, which would impact the physiological characteristics and growth of the plant (Munns et al., 2006). PGPRs alleviate the deleterious effects of salt stress by reducing the sodium absorption, as well as increasing the uptake of potassium in the leaves and roots, thereby maintaining an optimal K^+/Na^+ ratio for plant growth and induction of transcription factors under stress conditions (Karlidag et al., 2013). Nunkaew et al. (2015) indicated that exopolysaccharide producing halotolerant PGPRs can decrease plant sodium accumulation via binding Na^+ in roots and preventing its translocation to leaves (Ashraf et al., 2004; Dodd & Perez-Alfocea, 2012; Etesami & Beattie, 2017; Qin et al., 2016). The produced polysaccharides can also chelate free Na^+ in the soil making it unavailable to plants (Liu et al., 2022). Zhang et al. (2022) reported that EPS production by rhizobacteria was induced by root exudates to support mature biofilm formation. EPS are involved in cell–cell aggregation, which is essential for bacteria anchoring and adhesion to plant roots (Bhagat et al., 2021). Therefore, EPS presence improves biofilm formation and root colonization by salt-tolerant plant growth-promoting rhizhospheric bacteria, which may impact salt resistance in plants (Bhagat et al., 2021). Sun et al. (2022) found that EPS production by bacteria around roots increases leaf water potential and improves plant nutrient uptake. Furthermore, EPS enhance soil physicochemical properties and promote its aggregate formation (Kumar et al., 2020). Thus, EPS-producing PGPR can play a significant role in alleviating salinity in plants (Bhagat et al., 2021).

P. australis response to salinity is well documented, but the likely beneficial role of inoculation with halotolerant PGPR to improve this plant performance under saline conditions has still not been addressed. Therefore, the present study aims to (i) investigate the effectiveness of *Glutamicibacter* sp. to improve plant growth of *P. australis* under salt stress condition and (ii) identify some of the physiological and biochemical mechanisms modulated by this strain by emphasizing nutritional and biochemical traits (polyphenol, total soluble sugars [TSSs], proline, and markers of oxidative stress) of plants challenged or not with high salinity.

2 | MATERIALS AND METHODS

2.1 | Plant and microorganism sampling and characterization

Native *P. australis* rhizome sections were collected from reed populations in Borj Cedria (20 km to the east of Tunis, Tunisia). The PGPR strain *Glutamicibacter* sp. was isolated from rhizospheric soil from the salt-affected area in Soliman Sebkha (36 42′35″N 10 26′08″E, 30 km south of Tunis, semi-arid bioclimate) and was selected on the basis of its plant growth-promoting attributes and its EPS, siderophore, and IAA production.

EPS production by *Glutamicibacter* sp. was determined during the growth of this species when cultivated under increasing gradient of NaCl concentrations (0.1–1 M). Briefly, 150 mL flasks containing 50 mL of yeast extract mannitol (YEM) medium were inoculated with 0.1 mL of 10^8 colony-forming unit (CFU) bacterial culture and incubated at 28°C with shaking (180 rpm) for 3 days (Dueñas et al., 2003). Another experiment was performed by cultivating the strain in YEM medium without NaCl (control). After incubation, EPS yield was determined using the phenol-sulfuric acid method (Kazy et al., 2002). All experiments were performed in triplicate.

Bacterial strain was also assessed for its ability to survive under salt stress. The isolate was inoculated on Luria-Bertani (LB) liquid medium supplemented with different NaCl concentrations (0.1–1 M) and incubated at 28 ± 2°C with shaking at 170 rpm for 48 h. Control medium was maintained with 0.1% NaCl (w/v). Turbidity was determined by measuring the optical density (OD) at 600 nm. All experiments were performed in triplicate.

Identification of the bacterial strain was performed by the isolation of genomic DNA as described by Pospiech and Neumann (1995) followed by polymerase chain reaction (PCR) amplification of 16S rRNA as described by Weisburg et al. (1991). Partial sequence obtained was matched against nucleotide sequence present in GenBank using the BLASTn program (http://www.ncbi.nlm.nih.gov) and deposited in the GenBank database under accession number MK847918.

2.2 | Pot experiment and *P. australis* plant growth parameters

Several sections (approximately 5 g each) of *P. australis* rhizomes were inserted into 4-L pots containing autoclaved limono-sandy soil (pH 6.65; electrical conductivity: 0.05 dS m^{-1}; 0.24 and 0.45 g kg^{-1} of dry soil of P_2O_5 and total N; 0.25, 0.95, 0.65, and 0.05 meq 100 g^{-1} of dry soil of Na^+, K^+, Ca^{2+}, and Cl^-, respectively). All rhizome sections had an apical bud. Pots were placed under daily irrigation with distilled water. After 70 days, sprouted *P. australis* rhizomes were transferred to 2-L pots (two plants per pot) filled with sterilized soil. Plants were then grown for 40 days under greenhouse conditions. Twenty pots of similar *P. australis* plants were selected and assigned numbers. In the appropriate pots, plants were inoculated with 1 mL of the bacterial culture (10^7 CFU mL^{-1}). Seven days after inoculation, plants were watered three times per week by a mixture of distilled water and a solution of 300 mM NaCl. NaCl was added gradually in four concentrations (50, 100, 200, and 300 mM) on alternative days to avoid an osmotic shock. Plants were maintained under these conditions for additional 20 days. The experiment consisted of a factorial design with two inoculation treatments: non-inoculated (control) and inoculated plants with *Glutamicibacter* sp. and two saline treatments: plants treated with 0 mM NaCl and plants treated with 300 mM NaCl. Five replicates per treatment were used.

The fresh weight (FW) and dry weight (DW) of shoots and roots were determined after counting leaves and the tiller number. The DW of shoots and roots samples were determined after an oven-drying of 1 week at 60°C.

2.3 | Inorganic ion assay

Inorganic ions were extracted by mineralization of shoot and root dry powder (30 mg) in 30 mL nitric acid (0.1 N). Cations (Na^+, K^+, and Ca^{2+}) were assayed by a Corning 480 flame photometer and chloride by coulometry using a Haake-Buchler chloridometer.

2.4 | Lipid peroxidation

The extent of lipid peroxidation was calculated by measuring the malondialdehyde (MDA) content formed through thiobarbituric acid (TBA) reaction following the method of Halliwell and Gutteridge (1989). Lipid peroxides were extracted by grinding 0.05 g of shoot with 2 mL of trichloroacetic acid (TCA) 0.1% (w/v). The homogenate was centrifuged at 12,000 ***g*** for 20 min, and then 1 mL sample of the supernatant was mixed with 1 mL of 0.5% (w/v) TBA in 20% (w/v) TCA. The mixture was incubated at 95°C for 30 min; the reaction was stopped by placing the reaction tubes in an ice water bath. Following cooling, the sample was centrifuged at 5000 ***g*** for 5 min, and the supernatant was read at 532 and 600 nm. The concentration of MDA was calculated from the extinction coefficient 155 mM^{-1} cm^{-1}.

2.5 | Determination of organic solutes in plant tissues

Shoot free proline content was spectrophotometrically quantified by the ninhydrin method (Bates et al., 1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and centrifuged at 20,000 ***g***. Samples were incubated with 1 mL of ninhydrin acid and 1 mL glacial acetic acid and boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 2 mL of toluene, and absorbance was read at 520 nm. The proline content in each sample was calculated from a standard curve prepared with L-proline.

TSS content was estimated by the method of Yemm and Willis (1954). A sample of 25 mg of dry material was extracted in 80% ethanol solution. The extract was incubated for 30 min at 70°C and then centrifuged at 3000 ***g*** at 25°C for 30 min. TSS were analyzed by reacting 0.5 mL of the alcoholic extract with 5 mL freshly prepared anthrone and 2 mL ethanol (80%) and heated in a boiling water bath for 10 min. A standard curve was determined using glucose. After cooling, the absorbance was read at 640 nm with a spectrophotometer.

2.6 | Estimation of total polyphenol content

Phenolic compounds were assayed using the Folin–Ciocalteu reagent, following the method of Singleton and Rosi (1965) slightly modified by Dewanto et al. (2002). Shoot extracts were obtained by stirring 1 g of dry powder with 10 mL of pure methanol for 30 min. Sample extract was added to deionized water and Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min before adding 7% sodium carbonate solution and adjusted with distilled water. After incubation in the dark for 90 min at ambient temperature, the absorbance was measured at 760 nm. Gallic acid was used to prepare standard curve, and results were expressed as mg gallic acid equivalent (GAE) per gram of DW (mg GAE g^{-1} DW).

2.7 | Statistical analysis

Statistical analysis was performed using SPSS 20.0 statistical program (SPSS Inc., Chicago, IL, USA). The effects of the experimental factors, salt stress, and inoculation as well as the effect of their interactions on plant parameters were assessed by a two-way analysis of variance (ANOVA) (Table 1), and means were compared according to Duncan's test at $P < .05$. Principal component analysis (PCA) and correlation analysis were performed using XLSTAT software v. 2014.

3 | RESULTS

3.1 | Optimum NaCl tolerance of *Glutamicibacter* sp. and production of EPS in different NaCl concentrations

Glutamicibacter sp. showed the highest growth rate in the 100–200 mM NaCl salinity range, before progressively decreasing with increasing salt concentration (Figure 1a). EPS production by several halotolerant bacteria is frequently reported as a growth strategy that can form a protective biofilm around cells, which could protect them from oxidative damages under stress conditions. Therefore, we examined the potential role of EPS in salt tolerance of *Glutamicibacter* sp. As shown in Figure 1b, *Glutamicibacter* sp. produced EPS at various salt concentrations. EPS yield produced by *Glutamicibacter* sp. decreased at first with increasing salt concentration. This parameter showed a decrease of 14% and 36.5% at 200 and 400 mM NaCl compared to salt-free conditions and then remained stable regardless of salt concentration.

3.2 | Inoculation effect on plant growth

P. australis plants showed significant reduction in growth traits and biomass in response to NaCl stress, but inoculation with *Glutamicibacter*

TABLE 1 Two-factor ANOVA (bacterial inoculation and saline stress) for all parameters studied of *P. australis*; *F*-values (*P*-values).

	Bacterial inoculation (M)	Saline stress (S)	Interaction (B × S)
SFW	11.79 (.009)	157.27 (<.001)	0.413 (.538)
SDW	7.345 (.024)	29 (<.001)	1.41 (.266)
RFW	4.802 (.060)	247.86 (<.001)	8.043 (.022)
RDW	2.958 (.124)	33.11 (<.001)	4.247 (.073)
TN	6.658 (.024)	11.836 (.005)	2.959 (.111)
SN	5.014 (.052)	28.369 (<.001)	1.044 (.334)
Polyphenols	44.291 (<.001)	66.028 (<.001)	0 (.999)
Proline	1.699 (.229)	6027 (<.001)	30.078 (.001)
TSS	16.665 (.004)	9.801 (.014)	9.298 (.016)
MDA	19.928 (.001)	16.940 (.002)	0.113 (.743)
S Na^+	1.923 (.203)	93.071 (<.001)	20.116 (.002)
R Na^+	1.413 (.265)	1228 (<.001)	3.630 (.089)
S K^+	38.11 (<.001)	0.783 (.402)	0.084 (.780)
R K^+	8.089 (.022)	9.203 (<.016)	8.067 (.022)
S K^+/Na^+	2.654 (.134)	58.843 (<.001)	14.047 (.004)
R K^+/Na^+	4.415 (.065)	219.381 (<.001)	6.910 (.027)
S Cl^-	11.174 (.007)	436 (<.001)	18.169 (.001)
R Cl^-	3.600 (.087)	1590 (<.001)	20.969 (.001)
S Ca^{2+}	1.550 (.241)	0.035 (.856)	21.876 (.001)
R Ca^{2+}	51.598 (<.001)	15.935 (.003)	1.506 (.248)

Abbreviations: LN, leaves number; MDA, malondialdehyde; R Ca^{2+}, root calcium content; R Cl^-, root chloride content; RDW, root dry weight; RFW, root fresh weight; R K^+, root potassium content; R Na^+, root sodium content; S Ca^{2+}, shoot calcium content; S Cl^-, shoot chloride content; SDW, shoot dry weight; SFW, shoot fresh weight; S K^+, shoot potassium content; S Na^+, shoot sodium content; TN, tillers number; TSS, total soluble sugar.

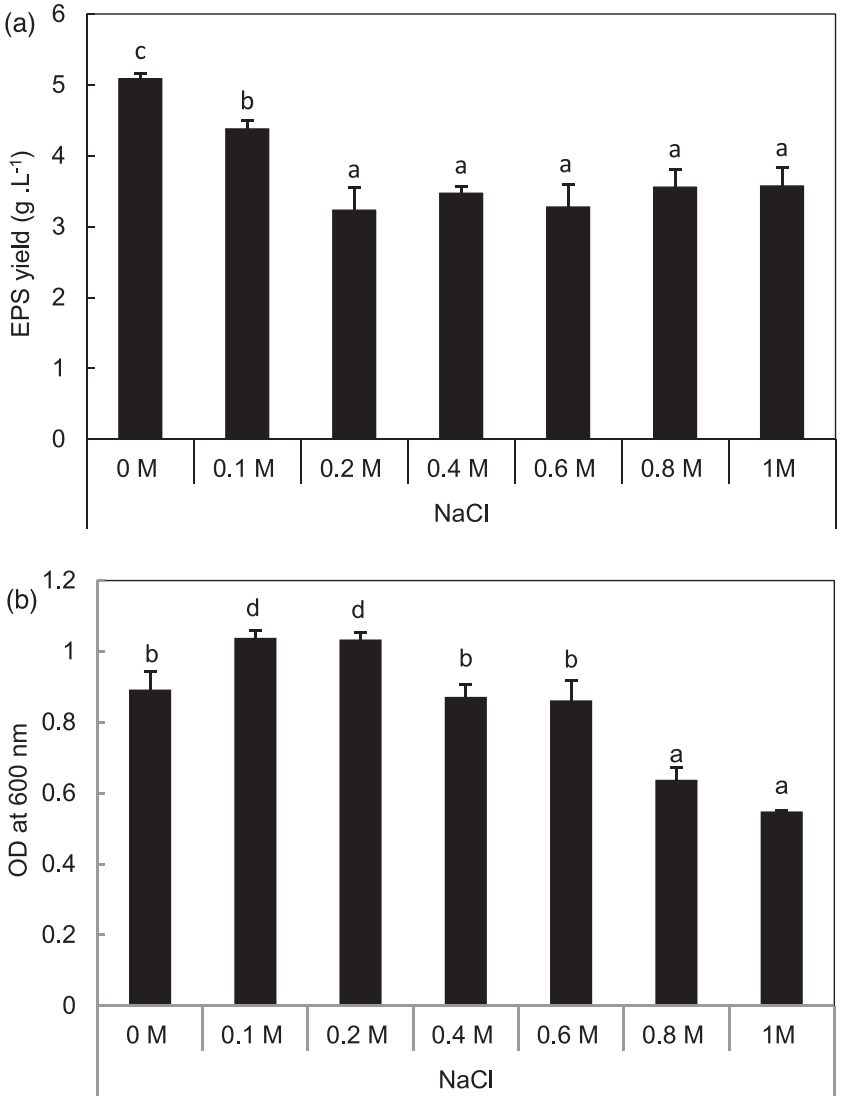

FIGURE 1 Determination of the growth rate and exopolysaccharides yield production of *Glutamicibacter* sp. in different saline levels (0.1–1 M NaCl). Different letters indicate statistically differences between salt concentrations (Duncan's least significant difference, $P < .05$, $n = 3$).

sp. isolate significantly (at $P < .05$) improved plant growth compared to non-inoculated stressed plants (Figure S1). *P. australis* inoculation with *Glutamicibacter* sp. strain in the presence of NaCl stimulated shoot FW and DW (+40% and +56%, respectively) (Figure 2a,b), root FW (+56%) (Figure 2c), and leaf and tiller numbers (+56% and +33%, respectively) (Figure 2e,f), when compared to non-inoculated plants challenged with salinity. It is worth mentioning that under salt-free conditions, the inoculation with *Glutamicibacter* sp did not significantly affect *P. australis* growth, except for root DW (approximately +17% as compared to non-inoculated plants) (Figure 2d).

3.3 | Inoculation effect on plant ion status

Under salt stress conditions, Na^+ concentration in shoot and especially root of non-inoculated plants increased by 90% and 270% as compared to the control plants, respectively (Table 2). This is consistent with the exclusive character of the plant when salt-challenged. *Glutamicibacter* sp. inoculation improved plant capacity to avoid Na^+ build-up in shoots, as reflected by the significantly lower shoot Na^+ concentration under salt stress (−20% compared to non-inoculated stressed plants) (Table 2).

Generally, no significant difference was found with respect to shoot and root K^+ concentration between non-inoculated control plants and non-inoculated stressed plants (Table 2). Yet, *Glutamicibacter* sp. significantly increased shoot K^+ concentration under non-salt stress as compared with control and salt-stressed plants (~30% on average). Under stress condition, *Glutamicibacter* sp. significantly enhanced K^+ concentration in root as compared to control (Table 2). Besides, the K^+/Na^+ ratio was enhanced by *Glutamicibacter* sp. in shoot of salt challenged plants (Table 2). *Glutamicibacter* sp. inoculation significantly decreased root Cl^- content by 12% under salt condition, as compared to salt-stressed plants only (Table 2). Calcium accumulation was higher in root than in shoot in inoculated and non-inoculated plants (Table 2). Root Ca^{2+} concentration was significantly higher in inoculated plants compared to non-inoculated plants under both saline and non-saline conditions (Table 2).

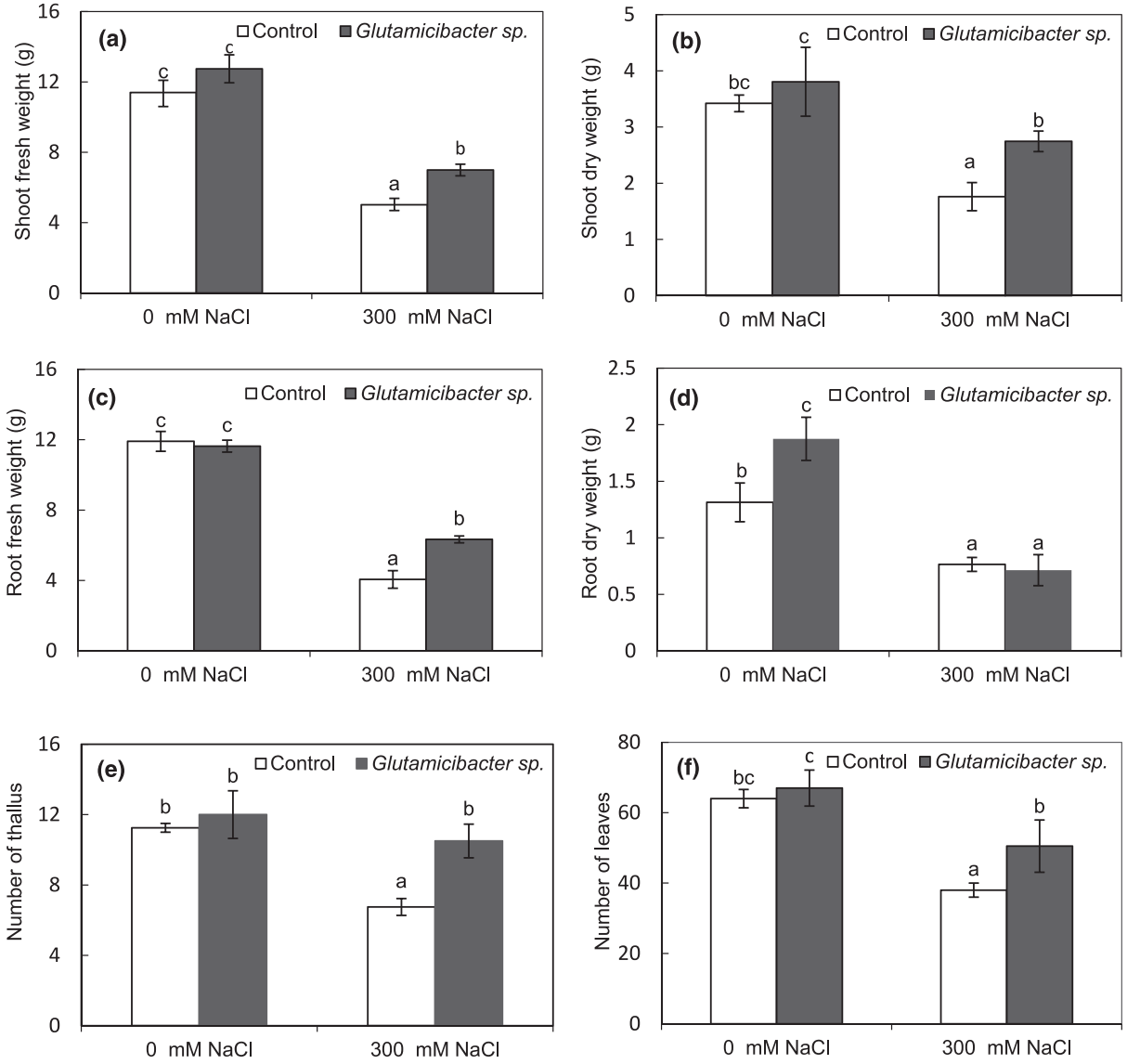

FIGURE 2 Effect of *Glutamicibacter* sp. on growth of *P. australis* under salt stress (300 mM NaCl): shoot fresh weight (a), root fresh weight (b), shoot dry weight (c), root dry weight (d), leaf number (e), and tiller number (f). Values are the means standard deviation of five replicates. Different letters indicate statistically differences between treatments (Duncan's least significant difference, $P < .05$, $n = 5$).

3.4 | Inoculation effect on lipid peroxidation and total polyphenol content

Lipid peroxidation level of *P. australis* plants were significantly increased by rising NaCl concentration. *Glutamicibacter* sp. significantly reduced this parameter under both non-saline and salt stress conditions; the decrease amounted to ~17% in both conditions (Figure 3a). Leaf total polyphenol content was higher with increasing concentration of salt (36% compared to the control) (Figure 3b). Interestingly, inoculated plants showed higher leaf polyphenol content than non-inoculated plants irrespective of salinity level (Figure 3b).

3.5 | Effect of *Glutamicibacter* sp. inoculation on free proline and TSSs content

Under salt-free conditions, no significant difference of leaf TSSs and proline content was observed between control and *Glutamicibacter* sp. treatment (Figure 4a,b). In non-inoculated plants, the TSSs and proline contents were increased by salt as compared to the control (Figure 4a,b). However, inoculation with *Glutamicibacter* sp. could significantly offset this effect by maintaining the TSS content in salt-treated plants and decreasing proline content by 30% as compared to salt-stressed plants only (Figure 4b).

TABLE 2 Effect of bacterial inoculation on nutrient accumulation in shoots and roots tissues of *P. australis* (mg/g DW): sodium (Na^+), potassium (K^+), chloride (Cl^-), and calcium (Ca^+) and K^+/Na^+ ratio in the absence (0 mM NaCl) and in presence of NaCl (300 mM NaCl).

Treatments	Nutrients									
	Na^+		K^+		Cl^-		Ca^{2+}		K^+/Na^+	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	11.35 ± 0.20a	12.8 ± 0.38a	23.18 ± 0.81a	13.11 ± 0.52a	12.94 ± 0.72a	12.37 ± 0.57a	9.84 ± 0.17b	22.5 ± 0.24a	2.14 ± 0.03c	0.91 ± 0.09b
Glutamicibacter sp.	13.57 ± 0.22a	13.47 ± 0.27a	29.8 ± 2.59b	13.11 ± 0.52a	17.64 ± 0.50b	15.25 ± 0.46a	9.28 ± 0.10a	30.06 ± 0.53b	2 ± 0.19c	0.81 ± 0.09b
300 mM NaCl	21.5 ± 1.92c	47.4 ± 0.37b	23.85 ± 0.93a	13.25 ± 1.45a	28.6 ± 0.54c	60.01 ± 2.29c	9.1 ± 0.23a	25.98 ± 2.66a	1.38 ± 0.07a	0.36 ± 0.02a
Glutamicibacter sp. + 300 mM NaCl	17.28 ± 0.64b	44.51 ± 3.32b	31.12 ± 1.31b	17.25 ± 1.08b	28.02 ± 0.50b	53.08 ± 0.50b	10.19 ± 0.12b	36.73 ± 1.25c	1.74 ± 0.05b	0.37 ± 0.03a

Note: Values are the means standard deviation of five replicates. Values sharing the different letters indicate significant differences between treatments at the 5% level (Duncan's *t*-test).

3.6 | Correlation analysis and PCA

For a more accurate interpretation of our data, a PCA (Figure 5) and correlation analysis (Table 3) were performed, taking into account all determined parameters characterizing plants under salt stress and bacterial inoculation. Globally, these analyses confirmed the observed positive effect of *Glutamicibacter* sp. inoculation, enabling *P. australis* to successfully cope with salt stress.

Positive correlations were found under *Glutamicibacter* sp. inoculation for shoot and root DWs and shoot FW. By contrast, this treatment was found to be negatively correlated with MDA content and root Na^+ content (Figure 5 and Table 3). A high salinity condition was negatively correlated with plant growth-related traits (shoot DW/FW and root FW, leaves number, and tillers number) (Figure 5 and Table 3). Besides, salt treatment was positively correlated with proline, MDA, TSS, Na^+, and Cl^- contents. Positive correlations were found under *Glutamicibacter* sp. inoculation under salt stress for polyphenols and shoot/root K^+ and Ca^{2+} contents. Finally, the results obtained by PCA and correlation analysis showed a perfect match with our trait-by-trait analyses.

4 | DISCUSSION

Due to their sessile state, plants are unavoidably affected by and respond to external biotic and abiotic stresses. So far, plants have to confront the stresses and develop diverse mechanisms of adaptation to avoid or tolerate their adverse effects so as to endure and to survive (Li et al., 2021). Soil salinization is one of the major environmental stressors that adversely impact plant growth and development in affected regions globally (Sunita et al., 2020). In order to adapt plant growth and salt stress tolerance, it is urgent to adopt new sustainable and environmental-friendly agricultural practices. The application of halotolerant PGPRs is widely recognized as an effective biological strategy for mitigating the harmful effects of high salinity in plants. It is well documented that some salt-tolerant bacteria and their extracellular polymers such as EPS can alleviate the adverse effects of salinity stress in plants by regulating plant cell physiological conditions (Sharma et al., 2016; Sun et al., 2022).

In the present study, we screened halotolerant rizhobacterium that produced a large amount of EPS, which were helpful to tolerate abiotic stress. EPS production is an important criterion for the classification of stress-tolerant microbes. Bhagat et al. (2021) indicated that EPS promotes bacterial survival under stress conditions by providing carbon source and enhancing water retention. Therefore, EPS helps in the establishment of plant–bacteria interactions by providing a microenvironment in which bacteria can survive under stress conditions (Bhagat et al., 2021). In the last few years, research showed that EPS production is highly beneficial for plants subjected to salt stress (Liu et al., 2022; Sun et al., 2022; Talebi Atouei et al., 2019). Microbial EPS act as a physical barrier in the soil protecting roots and promoting plant growth under high salinity conditions (Bhagat et al., 2021; Talebi Atouei et al., 2019). Besides, EPS production by bacteria in high

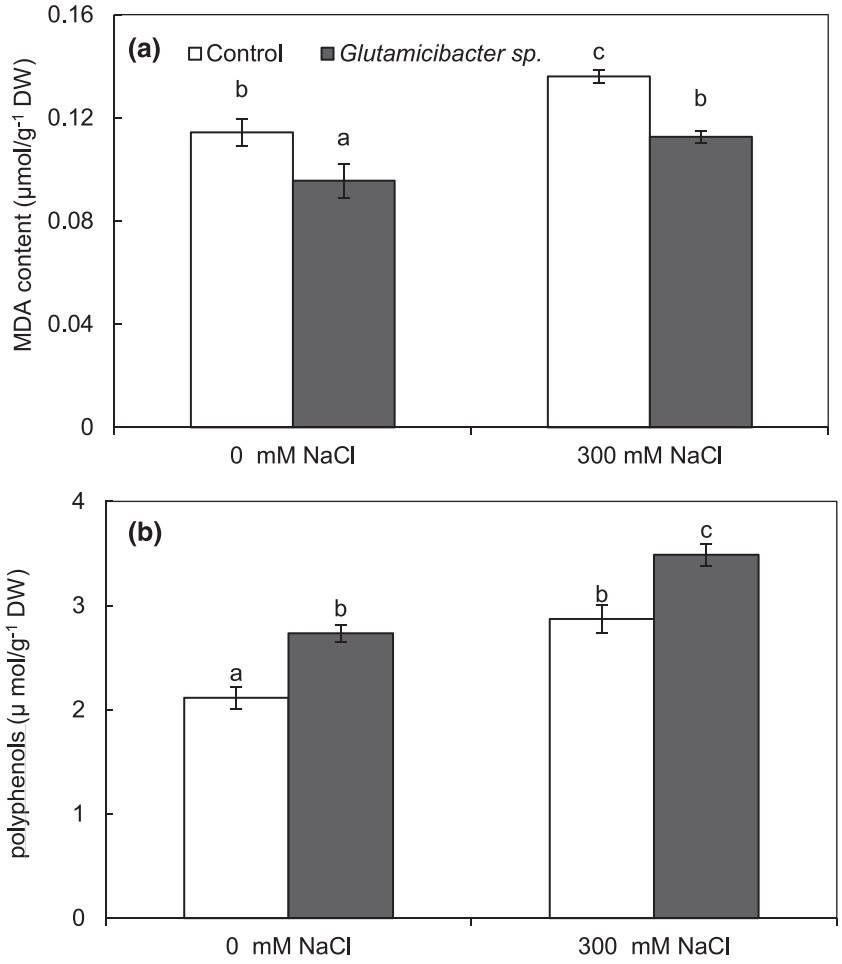

FIGURE 3 Effect of *Glutamicibacter* sp. on lipid peroxidation (a) and polyphenols content (b) in *P. australis* leaves under salt stress (300 mM NaCl). Values are the means standard deviation of five replicates. Different letters indicate statistically differences between treatments (Duncan's least significant difference, $P < .05$, $n = 5$).

salinity soil improves the quality, fertility, and stability of soil (Costa et al., 2018).

This study indicates that salinity significantly reduced shoot and root DWs of *P. australis*, which agrees with previously reported findings (Pagter et al., 2009). However, plants inoculated with *Glutamicibacter* sp. showed improved growth and hence better response than non-inoculated plants under salt stress conditions. Similar results were reported for salt tolerance in various plants induced by EPS-producing PGPR (Etesami & Beattie, 2017; Singh & Jha, 2017). *Glutamicibacter* species has been reported as an endophyte by various studies on tomato, potato, and maize (Afzal et al., 2019) and on halophytic species including the coastal halophytes *Limonium sinense*, *Cakile maritima*, *Matthiola tricuspidata*, and *Crithmum maritimum* (Christakis et al., 2021; Presta et al., 2016; Xiong et al., 2019). In contrast, several studies showed a co-occurrence of *Glutamicibacter* species as endophytic and rhizospheric strain (Khan et al., 2022). This could be due to the possible colonization of endophytes originating from the rhizospheric soil and later the colonization of roots and aerial organs of the plants.

Our findings indicate that salinity increases the plant uptake of Na^+ and Cl^-. According to previous literature, the strong accumulation of toxic ions (Na^+ and Cl^-) disturbs water balance and ion homeostasis, which then impacts other major physiological processes, such as hormonal status, transpiration, photosynthesis, translocation of nutrients, and other metabolic processes (Munns, 2002; Sharma et al., 2016; Santander et al., 2019).

By contrast, maintenance of intracellular ion homeostasis, particularly K^+ and Na^+ homeostasis, is one of the most powerful mechanisms involved by PGPRs for plants adapting to saline conditions (Munns & Tester, 2008). Indeed, PGPRs contribute to restricting the uptake and transport of Na^+ and Cl^- from the root to shoot and maintain plant mineral status. The K^+/Na^+ ratio clearly decreased in salt-treated plants due to the antagonism of Na^+ and K^+ at uptake sites in roots and the effect of Na^+ on K^+ transport into the xylem or inhibition of uptake processes (Hu & Schmidhalter, 2005). This is in agreement with a previous study pointing that inoculation with *Glutamicibacter* sp. may help plants to maintain ion homeostasis and high K^+/Na^+ ratio in shoots by reducing the uptake and/or transport of Na^+ and Cl^- to shoots and boosting the activity of high affinity K^+ transporters (Xiong et al., 2019). Reduced Na^+ concentration in *P. australis* stressed plants, due to *Glutamicibacter* sp. inoculation, may have also contributed to prevent accumulation of cellular Na^+ to toxic levels. There are several reports on lower Na^+ concentrations in plants inoculated with PGPR under salinity stress (Radhakrishnan & Baek, 2017; Sharma et al., 2016; Shukla et al., 2012; Singh & Jha, 2017; Upadhyay et al., 2011). In the present study, one may

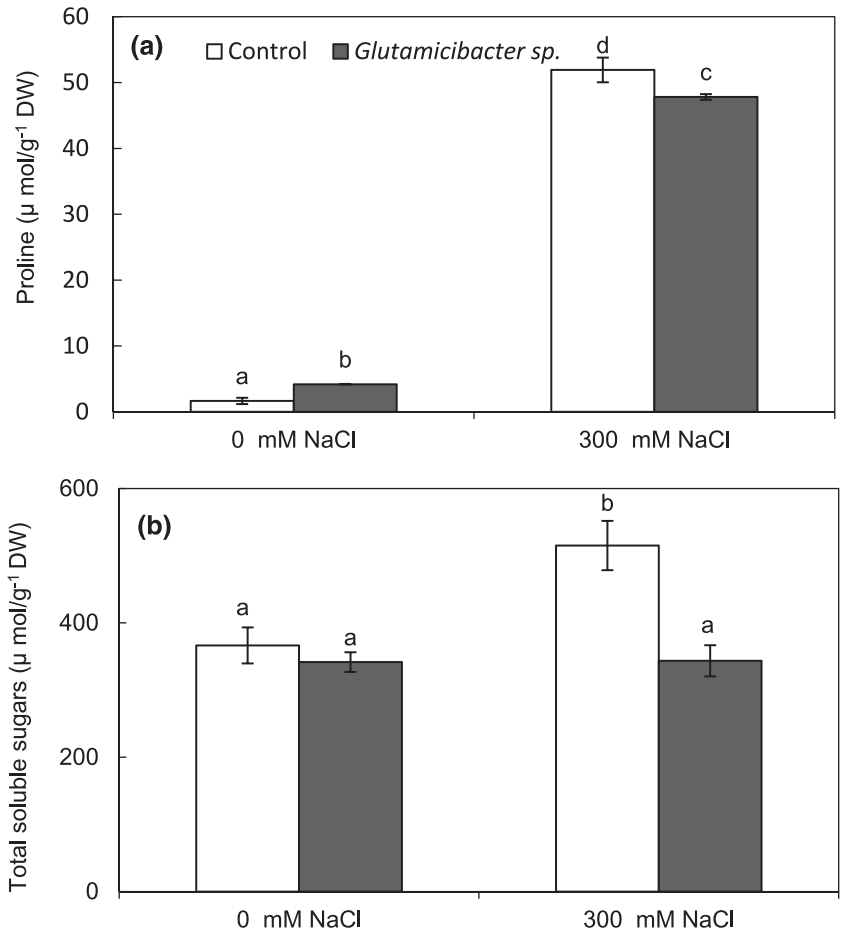

FIGURE 4 Effect of *Glutamicibacter* sp. on free proline (a) and total soluble sugars (b) in *P. australis* under salt stress (300 mM NaCl). Values are the means standard deviation of five replicates. Different letters indicate statistically differences between treatments (Duncan's least significant difference, $P < .05$, $n = 5$).

hypothesize that the excess of Na^+ resulting from the increasing levels of salinity might have been retained in the soil of inoculated plants, likely in relationship with *Glutamicibacter* sp.-produced EPS that bind cations, including Na^+, thus decreasing the content of Na^+ available for plant (Radhakrishnan & Baek, 2017). Hence, the reduction of Na^+ concentration in the leaves of inoculated *P. australis* could be due to a lower percentage of exchangeable Na^+ in the soil cultivated with such plants.

Inoculation with *Glutamicibacter* sp. showed a higher level of K^+ accumulation in *P. australis* plants might be the additional reason in the alleviation of salt stress. *Glutamicibacter* sp. increased plant Ca^+ uptake under saline conditions. Ca^+ is an essential second messenger that plays a vital role in salt signal transductions (Kader & Lindberg, 2010). Maintenance of high Ca^+ levels is a potential mechanism to alleviate the damages caused by the high concentrations of salts in soil (Yang et al., 2016).

Salinity impairs leaf net CO_2 assimilation rate by limiting photosynthesis, causing an over-depletion of photosynthetic electron chain, and redirecting the photon energy into processes that favor the production of reactive oxygen species (ROS) (Hichem et al., 2009; Johnson et al., 2003), which are considered as the key inducers of the programmed cell death in plants (De Pinto et al., 2012). Previous studies showed that oxidative damages and ROS generation caused by soil salinity were reflected in MDA content of plant leaves (Yazici et al., 2007). Therefore, peroxidation of membrane lipids is a useful indicator of salt-induced oxidative damages in membranes (Li et al., 2012). The present study revealed that growth reduction under saline condition in *P. australis* is associated with increased lipid peroxidation levels. However, there was a substantial reduction of the MDA content after plant inoculation with *Glutamicibacter* sp. suggesting that bacteria protects the plants from the imposed salt stress. In relationship to our findings, the lower level of lipid peroxidation is important in terms of salt tolerance as showed in different studies (de Azevedo Neto et al., 2006; Koca et al., 2007).

Polyphenol synthesis by plants is one of the adaptive mechanisms for reducing oxidative damages (Hichem et al., 2009; Nautiyal et al., 2008). In the present study, salinity significantly increased polyphenols' content in *P. australis* shoots, and bacterial inoculation also improved the amount of polyphenols. Polyphenols may eliminate radical species, thus preventing the propagation of oxidative chain reactions (Rice-Evans et al., 1997). Other studies also reported that inoculation with bacteria increased the content of polyphenols in maize leaves regardless of salt concentration (Rojas-Tapias et al., 2012). This strongly suggests that *Glutamicibacter* sp. improved *P. australis* tolerance through protecting plants from Na^+ toxicity and oxidative stress induced by salinity. Potassium also plays a major role in plant salt stress tolerance, notably to minimize oxidative cell damages, at least in part by reducing ROS formation during

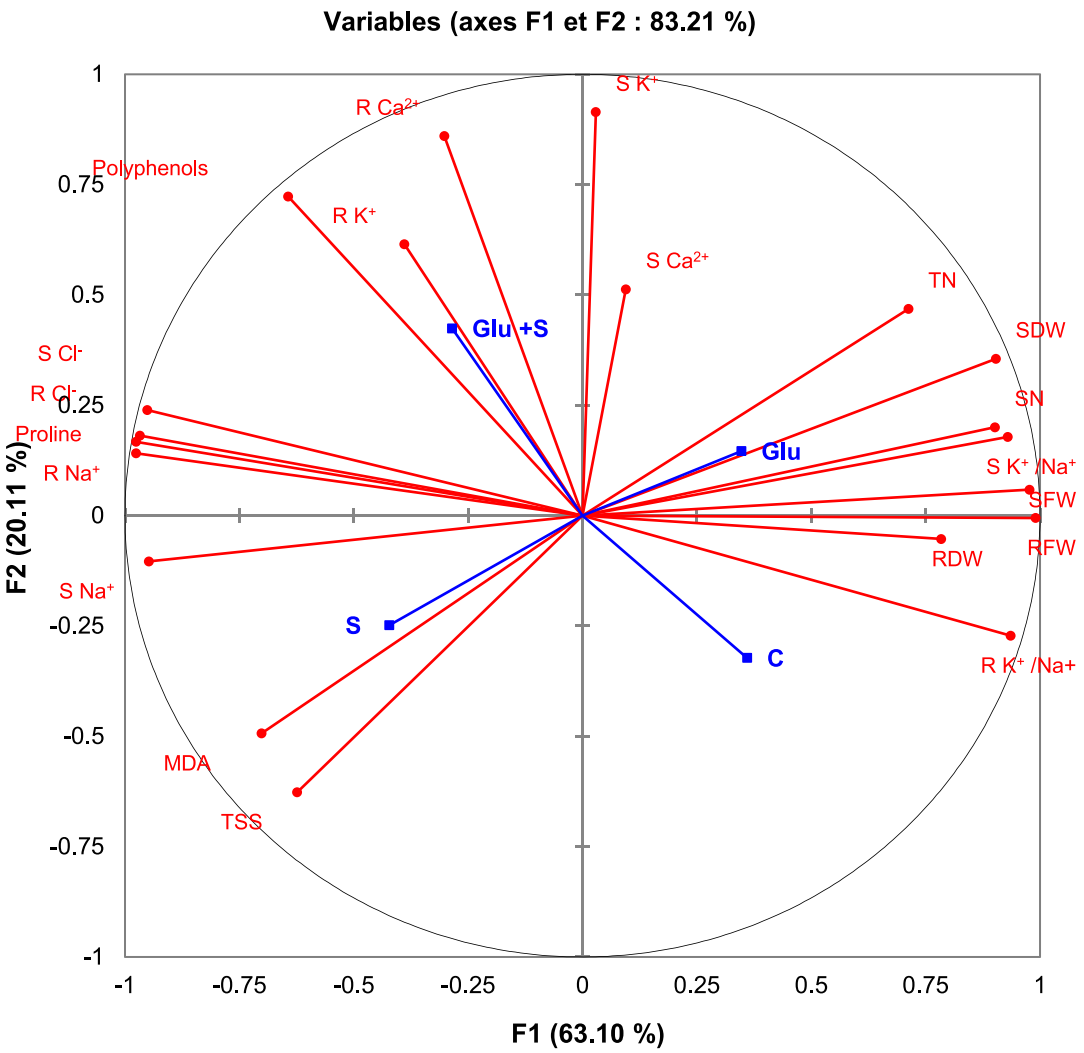

FIGURE 5 Principal component analysis (PCA). Circles (•) represent different analysis parameters. Squares (■) represent different treatments (C, Glu, S, and S + Glu). All studied parameters and the different treatments are projected onto the F1–F2 principal factorial plane that explains 83.21% of the variation. LN, leaves number; MDA, malondialdehyde; R Ca^{2+}, root calcium content; R Cl^{-}, root chloride content; RDW, root dry weight; RFW, root fresh weight; R K^{+}, root potassium content; R Na^{+}, root sodium content; S Ca^{2+}, shoot calcium content; S Cl^{-}, shoot chloride content; SDW, shoot dry weight; SFW, shoot fresh weight; S K^{+}, shoot potassium content; S Na^{+}, shoot sodium content; TN, tillers number; TSS, total soluble sugar.

photosynthesis and inhibiting activation of O_2^{-}-generating NADPH oxidase (Cakmak, 2005; Shen et al., 2000).

High salt concentrations in soil and water create a low osmotic potential, which reduces the availability of water to plants (Pérez-Romero et al., 2020). Osmotic adjustment by accumulation of solutes in plant cells in response to decreasing water potential in their environment partly contributes to maintain turgor pressure under these challenging conditions (Blum et al., 1996). Proline and TSSs are useful biochemical indicators of salinity tolerance in plants (Ashraf & Harris, 2004). Our results show that leaf concentrations of proline and TSSs increased with application of salt stress. It is reported that proline protects higher plants against salt/osmotic stresses, not only by adjusting osmotic pressure but also by stabilizing the structure of proteins and scavenging ROS under salt stress (Ashraf & Foolad, 2007). Under salt stress, PGPR-treated plants showed low proline content, but the level was higher than the basal level of proline in the control. This suggests that PGPR-treated plants do not face much salt stress; therefore, the proline accumulation is less. Nadeem et al. (2007) and Rojas-Tapias et al. (2012) similarly showed that plant proline contents are increased by saline stress, but decreased by inoculation with PGPR. Soluble sugars act as important osmolytes to maintain the cell homeostasis, where they constitute about 50% of the total osmotic potential in plant cells during salt stress (Gupta & Kaur, 2005); thus, increasing the TSS content could decrease osmotic potential in cells and maintain normal physiological function of plant cells in abiotic stress conditions (Bohnert & Shen, 1998). Similar to proline, the TSSs in inoculated plants are lower than in non-inoculated *P. australis* plants.

TABLE 3 Pearson's correlation matrix analyzing C, Glu, S, and S + Glu treatments and different studied parameters.

Variables	C	Glu	S	S + Glu
SFW	0.422	0.664*	−0.72**	−0.367
SDW	0.355	0.591*	−0.809**	−0.06
RFW	0.577*	0.532	−0.732**	−0.377
RDW	0.164	0.792**	−0.45	−0.506
TN	0.258	0.429	−0.773**	0.086
SN	0.299	0.609*	−0.753**	−0.213
Polyphenols	−0.763**	−0.086	0.082	−0.775**
Proline	−0.606*	−0.544	0.626*	0.525
TSS	−0.183	−0.363	0.896**	−0.349
MDA	−0.003	−0.705**	0.754**	−0.051
S Na^+	−0.663*	−0.341	0.808**	0.196
R Na^+	−0.517	−0.602*	0.637*	0.54
S K^+	−0.57	0.421	−0.47	0.619*
R K^+	−0.304	−0.303	−0.265	0.872**
S K^+/Na^+	0.655*	0.312	−0.838**	−0.111
R K^+/Na^+	0.738**	0.395	−0.507	−0.572*
S Cl^-	−0.716**	−0.432	0.566*	0.514
R Cl^-	−0.585*	−0.5	0.682**	0.512
S Ca^{2+}	0.285	−0.322	−0.617*	0.651*
R Ca^{2+}	−0.68**	0.184	−0.231	0.777**

Abbreviations: LN, leaves number; MDA, malondialdehyde; R Ca^{2+}, root calcium content; R Cl^-, root chloride content; RDW, root dry weight; RFW, root fresh weight; R K^+, root potassium content; R Na^+, root sodium content; S Ca^{2+}, shoot calcium content; S Cl^-, shoot chloride content; SDW, shoot dry weight; SFW, shoot fresh weight; S K^+, shoot potassium content; S Na^+, shoot sodium content; TN, tillers number; TSS, total soluble sugar.
*Statistically significant correlation at 0.05.
**Statistically significant correlation at 0.01.
***Statistically significant correlation at 0.001.

In conclusion, our data further confirm that PGPR can play a pivotal role in conferring salt tolerance in plants. EPS produced by *Glutamicibacter* sp. under saline condition plays a significant role in alleviating salinity stress because it can chelate free Na^+ from the soil and restrict Na+ entry into *P. australis* plants. *Glutamicibacter* sp. clearly improved plant salt tolerance through increasing plant antioxidative capacity and maintaining ion homeostasis, which strongly suggests that it may be useful to improve the growth of plants grown in saline alkaline soil.

AUTHOR CONTRIBUTIONS

Rabaa Hidri performed the experiments, analyzed data, and wrote the manuscript. Ouissal Metoui-Ben Mahmoud, Chedly Abdelly, and Rozario Azcon conceived and designed the study. Walid Zorrig contributed in data analysis. Ahmed Debez revised the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

PEER REVIEW

The peer review history for this article is available in the Supporting Information for this article.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supporting information; further inquiries can be directed to the corresponding author.

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