scientific reports

OPEN

Plant growth-promoting bacteria modulate metabolism and nitrogen accumulation to counteract drought damage in cactus pear plants

Gonçalves Albino Dauala[1], Bruno Sousa Figueiredo da Fonseca[2], Acacyara Batista de Sousa[1], Stelamaris de Oliveira Paula-Marinho[3], Pâmalla Graziely Carvalho Morais[1], Marcos Renan Lima Leite[3,4], Rodrigo Fonseca da Silva[1], Rhaiana Oliveira de Aviz[4], Maria Helena Ferreira Duarte[1], Romilda Rodrigues Nascimento[5], Elaine Martins da Costa[1], Cácio Luiz Boechat[1], Ademir Sérgio Ferreira de Araújo[1,4], Renato Oliveira de Sousa[1], José Hélio Costa[3], Ricardo Loiola Edvan[6], Francisco de Alcântara Neto[4,7] & Rafael de Souza Miranda[1,7]✉

Searching sustainable management techniques to enhance plant resistance to drought is crucial for agriculture in stressful scenarios. This study aimed to investigate the role of Plant Growth-Promoting Bacteria (PGPB) strains on forage cactus pear performance under drought. Trials were conducted in greenhouse using forage cactus plants in randomized block experimental design, 8 × 2 factorial scheme, consisting of eight inoculation treatments [inoculation with *Paenibacillus* sp. IPACC38 and IPACC55, and *Bacillus subtilis* IPACC29 strains combined with and without nitrogen (N) fertilization] and two water regimes [control at 50% field capacity (FC) and drought at 25% FC]. Drought significantly decreased plant growth across all treatments, regardless of N/inoculation treatment. The best performance under drought was observed in plants grown without N and inoculated with IPACC29 and IPACC38 strains. Improved performance under drought was closely related to PGPB's capacity to provide N accumulation for strategic photosynthetic pigments content, resulting in better root growth and plant development. Our findings reveal that IPACC29 and IPACC38 are the most promising PGPB strains for inoculating forage cactus pear, activating important defense responses against drought. This system offers alternative for cultivating cactus under water restriction and reduced N availability to increase green forage biomass production in dry regions.

Keywords Nitrogen availability, *Opuntia cochenillifera*, Semiarid, Stress tolerance, PGPB inoculation, Water deficit

Drought is a global phenomenon that affects crops growth and productivity in arid and semi-arid ecosystems worldwide, due to low rainfall and high temperatures throughout a year[1,2]. Water limitation negatively influences biochemical and physiological processes in plants, depending on intensity and duration of stress, well as plant developmental stage, genotypes and species[3–5].

[1]Postgraduate Program in Agricultural Sciences, Campus Professora Cinobelina Elvas, Federal University of Piauí, 64900-000 Bom Jesus, Brazil. [2]Agronomic Engineering Course, Campus Professora Cinobelina Elvas, Federal University of Piauí, 64900-000 Bom Jesus, Brazil. [3]Department of Biochemistry and Molecular Biology, Federal University of Ceará, 60440-554 Fortaleza, Brazil. [4]Postgraduate Program in Agronomy, Campus Ministro Petrônio Portella, Federal University of Piauí, 64048-550 Teresina, Brazil. [5]Department of Animal Science, Federal University of Campina Grande, 58708-110 Campina Grande, Brazil. [6]Department of Animal Science, Federal University of Piauí, 64048-550 Teresina, Brazil. [7]Department of Plant Science, Federal University of Piauí, 64048-550 Teresina, Brazil. ✉email: rsmiranda@ufpi.edu.br

Long periods of drought may impose severe limitations on both native and cultivated species, such as grasses and legumes, causing the decrease in biomass and pasture for animals[6]. This environmental constraint represents one of the main challenges faced by small- and medium-scale producers in providing adequate animal feed[7]. In this scenario, cultivating cactus pear (*Opuntia cochenillifera*) emerges as an alternative under water scarcity conditions, as it can serve as a source of food, water and energy for ruminants and other animals.

Cactus pear is a live forage reserve associated with good palatability and satisfactory biomass production, exhibiting high water-use efficiency due to the Crassulacean Acid Metabolism (CAM) photosynthetic pathway[6,8]. However, despite cactus pear plants exhibit good performance in dry regions, prolonged drought periods can negatively impact on the crop, resulting in decrease of nocturnal stomatal conductance, malic acid accumulation, relative water content, chlorophyll content and cladode development[9,10]. Water scarcity immediately after cladode planting may lead to plant death, resulting in planting failures and productivity losses under rainfed condition[11].

Therefore, searching for sustainable and low-cost technologies to mitigate drought-related damage in cactus pear cultivation is crucial, particularly for small- and medium-scale farmers in semi-arid and arid regions. Thus, plant growth-promoting bacteria (PGPB) have emerged as a promising strategy for enhancing crop resilience and production of green forage biomass under drought stress, as well as promoting sustainable agriculture by reducing dependence on agricultural fertilizers[12,13].

PGPB colonize the endo-rhizosphere or rhizosphere and enhance drought tolerance through direct and indirect mechanisms[5,14]. Some studies have pointed out that plant-PGPB interactions can provide nitrogen for plants as well as promote important alterations in primary and secondary metabolism, while also regulating plant stress-responsive pathways[5,12,13,15–18]. In kentucky bluegrass (*Poa pratensis*), inoculation with *Bacillus subtilis* strains promoted greater growth and development compared with uninoculated turfgrass under drought stress[19]. Also, drought-stressed bean plants (*Phaseolus vulgaris*) displayed an important increase in growth, nitrogen content, and nodulation under inoculation with *Paenibacillus* sp. strains[12,20].

Although numerous reports have evidenced the beneficial role of PGPB in enhancing drought tolerance in various crops, their impact on cactus pear plants under drought conditions remain underexplored. Our investigative study aimed to evaluate the role of plant growth-promoting bacterial strains to enhance the drought tolerance of cactus pear (Miúda cultivar, *O. cochenillifera*), by evaluating plant growth, physiological parameters, and biochemical indicators.

Materials and methods

The experiments were carried out under greenhouse conditions, between March and September 2023, at the Campus Professora Cinobelina Elvas, Federal University of Piauí (UFPI) (9°04′45.6″ S, 44°19′37.9″ W and 277 m altitude), in Bom Jesus city, Piauí state, Brazil.

Growing conditions, experimental design and treatments

The trials were conducted in a randomized block experimental design, in an 8 × 2 factorial scheme, with four repetitions, totaling 64 experimental units. The first factor consisted of eight inoculation treatments [three PGPB strains [*Paenibacillus* sp. IPACC38 (IC38) and IPACC55 (IC55) and *Bacillus subtilis* IPACC29 (IC29)] associated with and without N, and two uninoculated controls (with and without N)], while the second factor consisted of two water levels [well irrigated – 50% of soil field capacity (FC), and drought stress – 25% FC].

The Miúda cultivar from *O. cochenillifera* Salm-Dyck, a cultivated species not protected by intellectual property rights, was selected alongside the inoculation treatments based on findings from preliminary trials[23]. The PGPB strains were obtained from sugarcane tissues (surface disinfected) and identified by Antunes et al.[21]. These strains were selected based on ability to act in different processes (indole-3-acetic acid production and biological N_2 fixation) and promote the growth of plant species such as corn and sorghum[22], and cactus pear[23].

Soil samples of a dystrophic Yellow Latosol (Oxisols) from the 0–20 cm layer were collected at the Alvorada do Gurguéia experimental farm of UFPI for chemical and physical analyses (Table S1). The fertilization was corrected as necessary using P_2O_5 (as simple superphosphate, 18%), K_2O (as potassium chloride, 60%), Cu (as copper sulfate, 26.23%), Zn (as zinc sulfate, 22.73%), and N (as urea, 45%), following recommendations for cactus pear crop[24,25]. The nitrogen was applied as 50 mg dm^{-3} (equivalent to 100 kg ha^{-1}), corresponding to 50% of a dose recommended (100 mg dm^{-3} corresponding to 200 kg ha^{-1}) for cactus pear fertilization[24]. All fertilizers were incorporated to the pots containing 9 dm^3 of soil prior to planting.

Uniform matrix cladodes of Miúda cultivar were collected from the UFPI' Agrostological Experimental Field. The cladodes were selected from 2-year-old plants free from pests and diseases, preferably selecting younger (tertiary) cladodes with similar age, weight, and morphological characteristics such as size, diameter, and thickness. Before planting, PGPB strains were reactivated and checked for purity on TSA (Tryptone Soy Agar) medium at 25%, pH 6.8. For inoculum preparation, the PGPB strains were cultivated in Erlenmeyer flasks containing 50 mL of liquid medium TSB (Tryptone Soya Broth) at 25%, pH 6.8, and incubated on a SL-180/DT shaker Table (120 rpm) at 28 °C for 72 h. Then, the cladodes were inoculated with 1.0 mL of TSB medium containing bacteria strains (at a concentration of approximately 1×10^8 UFC mL^{-1}), applied at the base of cladode.

The plant material was planted by sowing one-third of matrix length in the soil, one per pot. For uninoculated treatment, 1.0 mL of TSB uninoculated medium was applied for plants. The nitrogen fertilization at a rate of 50 mg dm^{-3} was split into two applications: the first dose of 25 mg dm^{-3} was applied 30 days after planting, and the second dose of 25 mg dm^{-3} at 60 days after planting, using urea (45% N) as N source. The environmental conditions inside the greenhouse throughout the experiment are presented in Fig. 1.

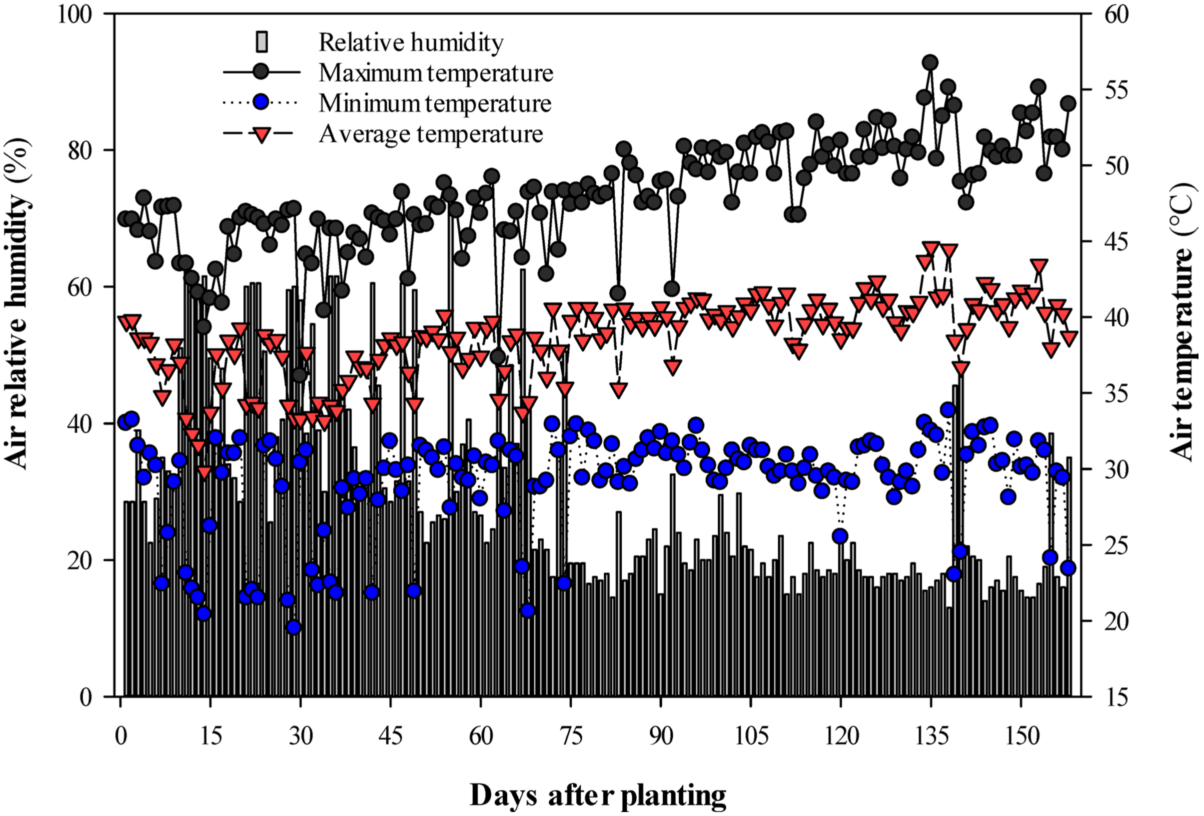


Fig. 1. Relative humidity and maximum, minimum and average air temperatures recorded in the greenhouse during the cultivation of cactus pear plants under different treatments.

Irrigation management

To ensure the stabilization of the matrix cladodes, soil humidity after planting was reached to 50% FC in all treatments, consisting a well-irrigated condition. The drought treatments were imposed 43 days after planting, by reducing the water level to 25% FC. The relative soil water content (RSWC) was daily monitored through gravimetric method using the following equation:

$$RSWC = \frac{(W_{soil} - W_{pot} - DW_{soil})}{(W_{FC} - W_{pot})}$$

where W_{soil} is the current soil weight, W_{pot} corresponds to weight of empty pot, DW_{soil} is the weight of dried soil, and W_{FC} correspond to the soil weight at field capacity (100%).

Weighing and watering were executed until the pots reached the RSWC targets for control (50% FC) and drought (25% FC) treatments. The plant material was harvested 193 days after planting, corresponding 150 days of drought imposition, and used for all analyzes.

Growth parameters and relative tolerance to drought

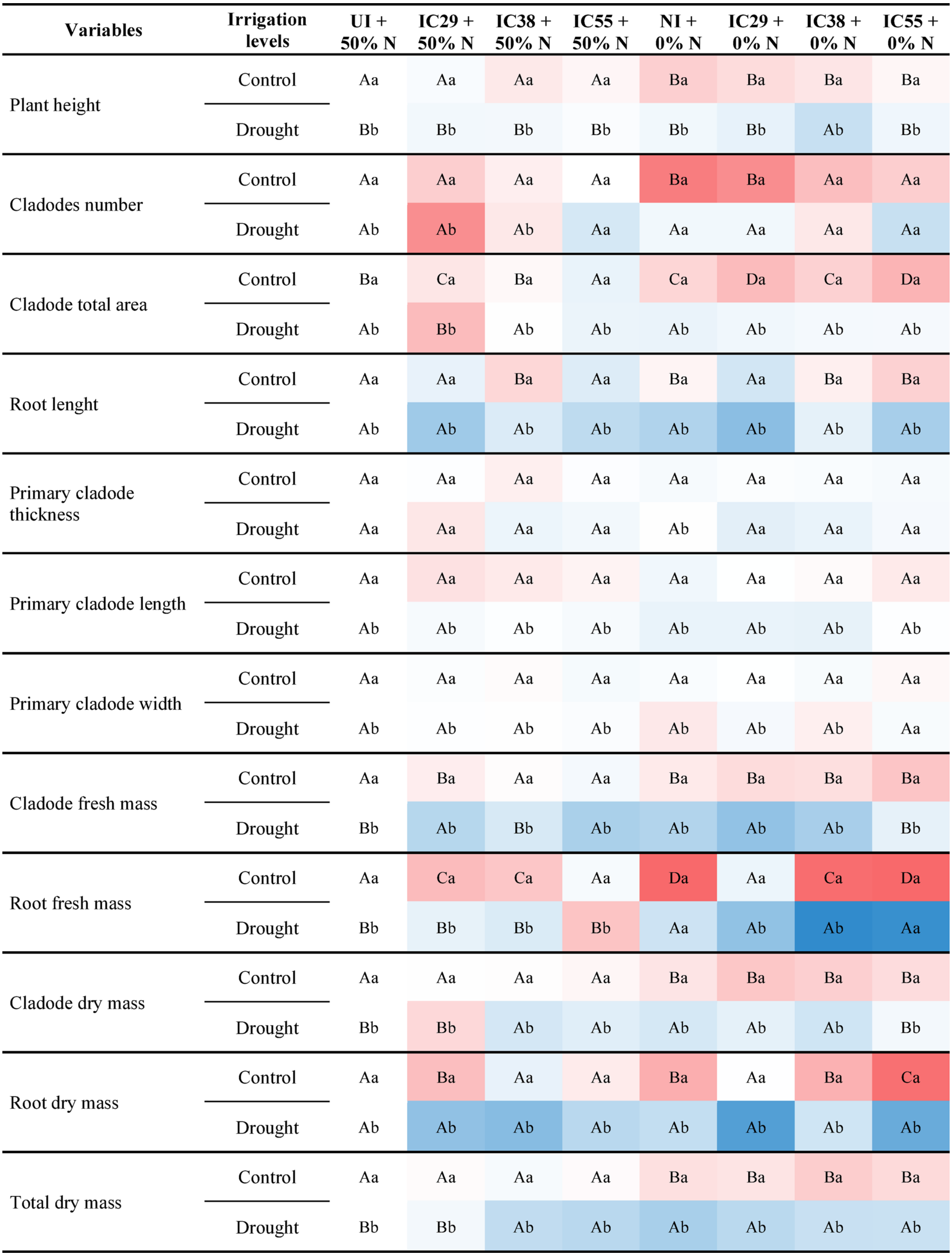
The harvest time-point was conducted 150 days after drought imposition, when plants exhibited visible symptoms and growth reduction due to water limitation. Non-destructive biometric parameters, including the number of cladodes per plant, plant height, and length and width of cladodes, were estimated. The thickness of primary cladodes was determined at the midpoint of the third cladode using a precision digital caliper. One representative plant per treatment was also selected for image recording.

The plants were separated into cladodes (matrix, primary and secondary) and roots to determine fresh mass and root length. Cladode area was determined using a digital area meter (LI-3100 C, LI-COR), and images of representative roots were acquired using a root scanner (FCLSD-0503, Delta-T Devices). Then, the material was immediately frozen and lyophilized to obtain the dry mass. The relative tolerance to drought was calculated as ratio between total dry mass of drought-stressed plants and total dry mass of control plants[26].

Cladode succulence and temperature

Cladode succulence (CS) was determined based on the following formula[27]:

$$\mathbf{CS}\ \left(\mathbf{g\,H_2O\,cm^{-2}}\right) = \frac{\mathbf{CFM - CDM}}{\mathbf{CA}}$$



Variables	Irrigation levels	UI + 50% N	IC29 + 50% N	IC38 + 50% N	IC55 + 50% N	NI + 0% N	IC29 + 0% N	IC38 + 0% N	IC55 + 0% N
Plant height	Control	Aa	Aa	Aa	Aa	Ba	Ba	Ba	Ba
	Drought	Bb	Bb	Bb	Bb	Bb	Bb	Ab	Bb
Cladodes number	Control	Aa	Aa	Aa	Aa	Ba	Ba	Aa	Aa
	Drought	Ab	Ab	Ab	Aa	Aa	Aa	Aa	Aa
Cladode total area	Control	Ba	Ca	Ba	Aa	Ca	Da	Ca	Da
	Drought	Ab	Bb	Ab	Ab	Ab	Ab	Ab	Ab
Root lenght	Control	Aa	Aa	Ba	Aa	Ba	Aa	Ba	Ba
	Drought	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Primary cladode thickness	Control	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa
	Drought	Aa	Aa	Aa	Aa	Ab	Aa	Aa	Aa
Primary cladode length	Control	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa
	Drought	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Primary cladode width	Control	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa
	Drought	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Aa
Cladode fresh mass	Control	Aa	Ba	Aa	Aa	Ba	Ba	Ba	Ba
	Drought	Bb	Ab	Bb	Ab	Ab	Ab	Ab	Bb
Root fresh mass	Control	Aa	Ca	Ca	Aa	Da	Aa	Ca	Da
	Drought	Bb	Bb	Bb	Bb	Aa	Ab	Ab	Aa
Cladode dry mass	Control	Aa	Aa	Aa	Aa	Ba	Ba	Ba	Ba
	Drought	Bb	Bb	Ab	Ab	Ab	Ab	Ab	Bb
Root dry mass	Control	Aa	Ba	Aa	Aa	Ba	Aa	Ba	Ca
	Drought	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Total dry mass	Control	Aa	Aa	Aa	Aa	Ba	Ba	Ba	Ba
	Drought	Bb	Bb	Ab	Ab	Ab	Ab	Ab	Ab

where CFM is the cladode fresh mass; CDM is the cladode dry mass; and CA is cladode area.

The temperature of cladodes (°C) was measured one day before harvest using an imaging IR thermometer FLIR (model TG165) starting from 01:00 pm.

Photosynthetic pigments

Photosynthetic pigments (chlorophylls *a*, *b*, total, and carotenoids) were extracted after incubating two 1.0 cm^2 discs of young cladodes in a dimethyl sulfoxide (DMSO) solution saturated with $CaCO_3$. The contents chlorophyll *a*, *b*, total and carotenoids were estimated in supernatant through spectrophotometry readings at

◀**Fig. 2**. Clustering analysis of growth and biomass parameters of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IC29), *Paenibacillus* sp. (IC38 and IC55) strains, 150 days after drought imposition. In all cases, blue color specifies an increase, and red denotes a decrease in the analyzed indexes, taking the data from uninoculated (UI) plants treated with 50% nitrogen as reference. White color represents no change. The varying intensities of red and blue express the extent of the change according to fold increase. For each assay, within the same row, different capital letters represent significant differences due to N/inoculation treatments; while, in the same column, different lowercase letters represent significant alterations due to drought stress, according to Scott–Knott's test ($p < 0.05$).

480, 649 and 665 nm[28]. The discs were then dried in a forced ventilation oven at 65 °C for three days until reaching a constant dry mass, and the pigments content were expressed as µg g^{-1} DM.

Nitrogen, soluble carbohydrates and free amino acids

The nitrogen (N) content (g kg^{-1}) in root and cladode tissues was determined using the Kjeldahl method[29]. Then, the N accumulation (g $plant^{-1}$) in plant tissues was calculated using the data from N content and dry mass[30].

Soluble carbohydrates and free amino acids were extracted by homogenizing powder lyophilized tissues in ethanolic solution (80% v/v) at 75 °C, for 1 h, with agitations each 15 min. Then, the homogenate was centrifuged at 3,000 x g for 10 min at 4 °C, and the resulting supernatant was collected, and the extraction process was repeated twice on the remaining precipitate. The soluble carbohydrates were estimated by absorbance readings at 490 nm, using standard curve of anhydrous D-glucose ranging from 0 to 250 ug mL^{-1}[31]. The free amino acids were measured by readings at 570 nm, using standard curve of L-glycine solutions ranging from 0 to 50 ug mL^{-1}[32]. The content of these organic compounds was expressed as µmol g^{-1} DM.

Statistical analysis

The data were initially submitted to normality analysis using the Shapiro-Wilk test at a 5% significance level; followed by an analysis of variance (ANOVA) using the F test at 5%. Then, significant interactions ($p \leq 0.05$) were submitted to the Scott-Knott mean test through Sisvar software version 5.6[33]. The hierarchical clustering was designed using Excel software, while the graphs were plotted through Sigma Plot 14.0 software. Finally, principal component analysis (PCA) and correlation network were conducted on the datasets analyzed through R software R (Development Core Team)[34].

Results

Plant growth, phenotypic appearance and root morphology

Under well-irrigated conditions, 50% N-treated plants exhibited plant height higher than those of 0% N treatments. The drought significantly decreased the height of plants from all treatments, irrespective of N/inoculation; however, the highest plant height was reported in IC38 + 0% N stressed plants as compared to other N/inoculation treatments (Fig. 2 and Table S2).

Alterations in cladode numbers due to N/inoculation treatments were observed only in well-irrigated plants, where the treatments without N inoculated with IC29 or without inoculation (UI) displayed low values as compared to other treatments. Drought stress promoted significant decrease in cladode number only in plants treated with 50% N, under inoculation with IC29 and IC38 and without inoculation, as compared to well-irrigated plants (Fig. 2).

The highest cladodes total area was found in well-irrigated plants from IC55 strain + 50% N. Drought stress severely decreased cladode total area and root length, and little or no alteration was observed due to N/inoculation treatments.

In general, the thickness, length and width of primary cladodes were not altered by N/inoculation treatments, but water limitation significantly decreased the length and width of primary cladodes in plants from all treatments. In addition, the fresh, dry and total mass of cladode and roots were found to be lower in plants growing without N, irrespective of inoculation. For all cases, drought promoted significant decrease in biomass of plant tissues, but the effects were less intense in inoculated plants without N (Fig. 2 and Table S2).

The phenotypic aspects of cactus pear at 150 days after drought imposition reveals more developed plants under well-irrigated conditions combined with 50% N, irrespective of inoculation treatment (Fig. 3). Interestingly, the drought-stressed plants from inoculated treatments without N exhibited visual characteristics closer to well-irrigated plants treated with 50% N.

Drought drastically impaired the development of cactus pear roots in plants from all N/inoculation treatments as compared to respective well-irrigated controls (Fig. 4). However, the inoculation with IC29 strain without N appear to alleviate the drought damage as compared to treatment without inoculation. Additionally, under drought, all treatments without N appeared in longer roots than those with 50% N (Fig. 4).

Water status and drought tolerance

Under control conditions, the cladode succulence was higher in plants from treatments without N as compared to those from 50% N treatments (Fig. 5A). Drought stress similarly decreased cladode succulence as compared to well-irrigated treatment, and no alteration due to N/inoculation was observed in water-stressed plants. The cladode temperature was increased by drought imposition as compared to well-irrigated control, with no significant alteration among the different N/inoculation treatments (Fig. 5B).

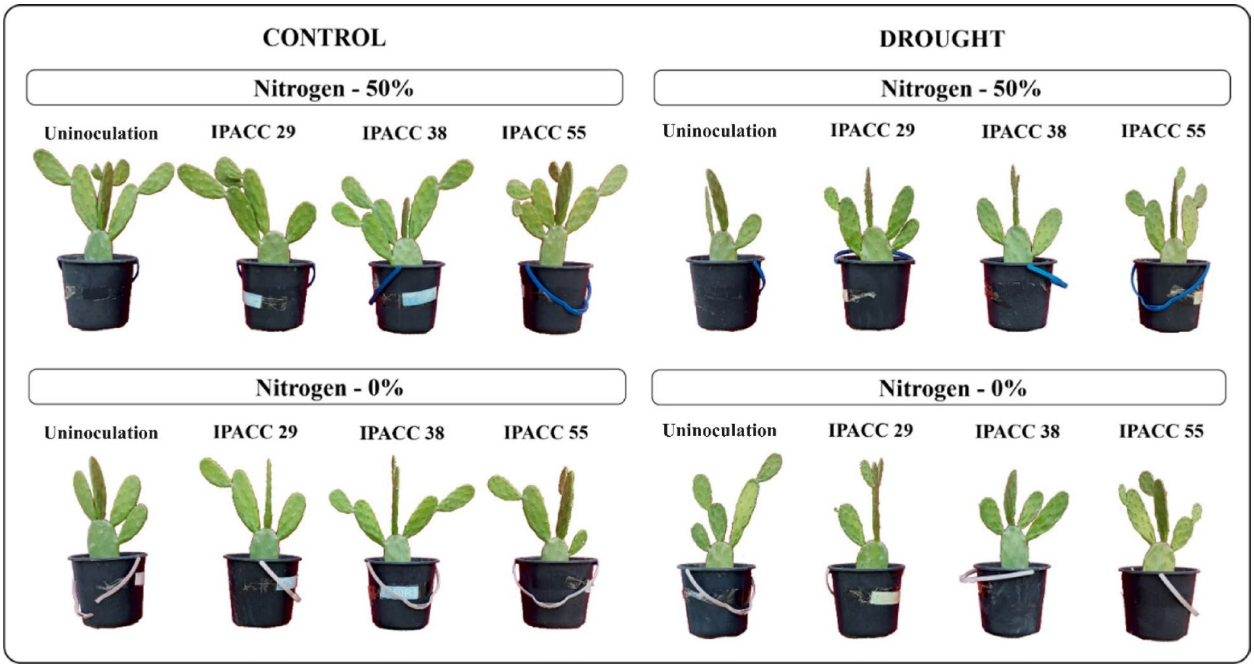


Fig. 3. Phenotypic appearance of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IC29) and *Paenibacillus* sp. (IC38 and IC55) strains, 150 days after drought imposition.

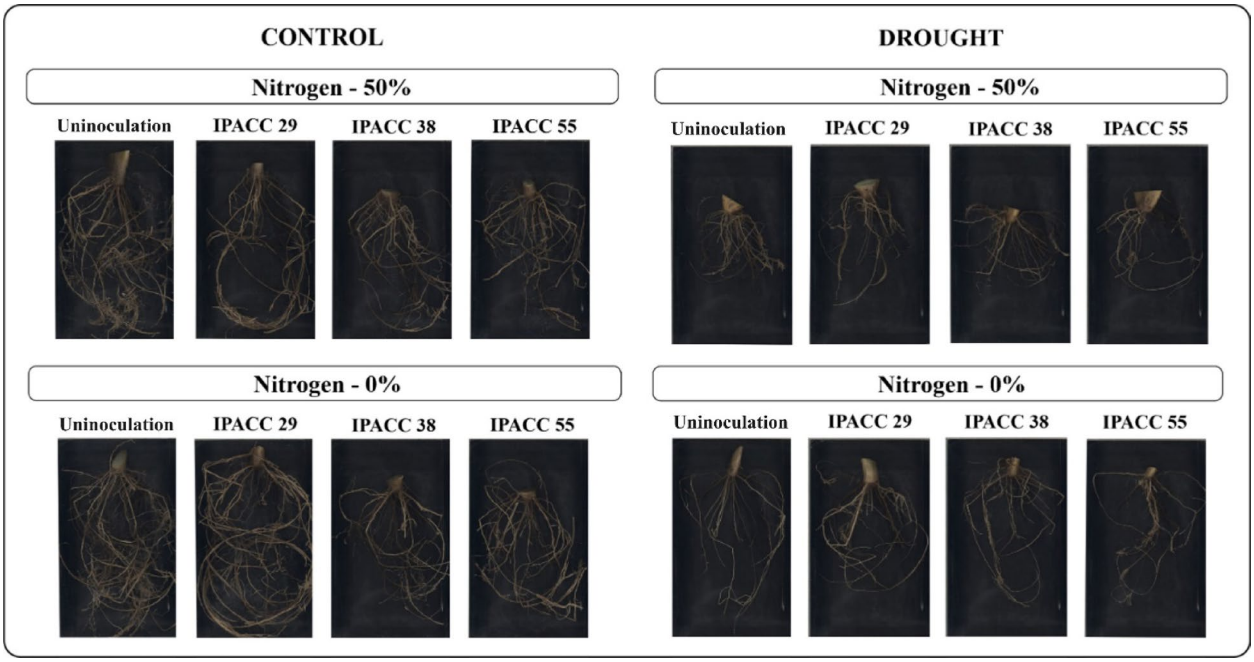


Fig. 4. Root morphology of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IC29) and *Paenibacillus* sp. (IC38 and IC55) strains, 150 days after drought imposition.

The negative impacts of drought in dry mass accumulation were reflected in decrease in relative drought tolerance, that was reduced in plants from all N/inoculation treatments. However, cactus pear plants exhibited higher drought tolerance without N (UI) and inoculated with IC29 and IC38 strains, as compared to other treatments (Fig 5C).

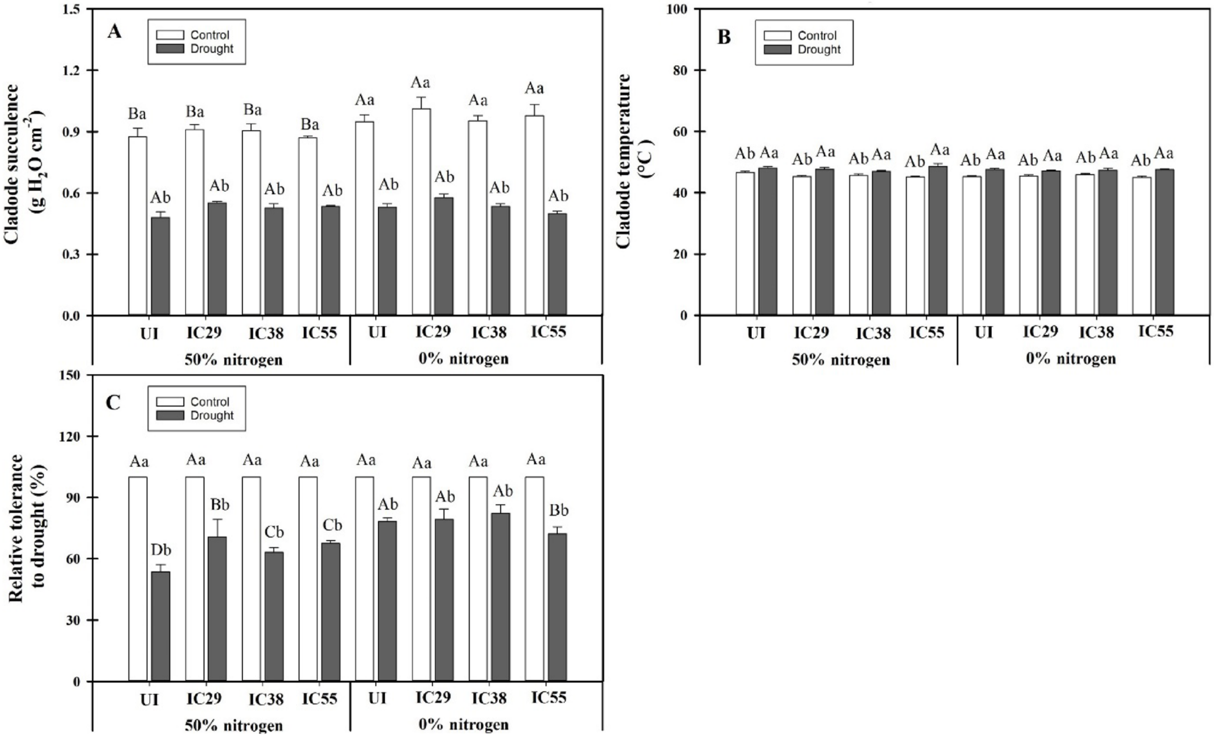


Fig. 5. Cladode succulence (**A**), cladode temperature (**B**), and relative tolerance to drought (**C**) of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IC29) and *Paenibacillus* sp. (IC38 and IC55) strains, 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to drought stress (Control × Drought), using Scott-Knott test ($p < 0.05$).

Nitrogen accumulation

In cladodes, under well-irrigated conditions, the nitrogen content and accumulation were higher in plants from 50% N treatments as compared to those without N (Fig. 6A, C). In 0% N treatment, the inoculation with IC29, IC38 and IC55 strains promoted significant increase in nitrogen content and accumulation compared to uninoculated plants (Fig. 6A, C). Drought promoted significant increase in nitrogen content of plants from all N/inoculations treatments, but a drought-induced increase in nitrogen accumulation was observed only in plants from 50% N uninoculated and inoculated with IC55, and in plants from 0% N uninoculated and inoculated with IC29 (Fig. 6A, C).

In roots, IC29-inoculated plants displayed elevated nitrogen content and accumulation under well-irrigated conditions, as compared to other N/inoculation treatments (Fig. 6B, D). Except for IC29-inoculated plants, drought stress promoted significant increase in nitrogen content in the roots, but in fact resulted in a reduced nitrogen accumulation in subterraneous tissues (Fig. 6B, D).

Photosynthetic pigments and organic compounds

The contents of photosynthetic pigments were similar in well-irrigated plants, irrespective of N/inoculation treatment (Fig. 7). Plants exposed to drought from all treatments showed significant increase in contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids as compared to well-irrigated control, with rare exceptions. Notably, the UI and IC38 treatments with 50% N, and the UI and IC55 treatments without N, showed the highest levels of chlorophyll *a* and total chlorophyll under deficit conditions (Fig. 7A, C). Yet, the highest chlorophyll *b* contents were found in uninoculated plants, regardless of N nutrition (Fig. 7B). Under drought, the highest carotenoids contents were observed in 50% N plants from UI and IC38 treatments (Fig. 7D).

The soluble carbohydrates and free aminoacids were differentially regulated as affected by both N/inoculation treatment and drought imposition (Fig. 8). The soluble carbohydrates content in cladodes was decreased by drought stress as compared to well-irrigated control, irrespective of N/inoculation treatment (Fig. 8). Conversely, in roots, the soluble carbohydrates were maintained unaltered or increased by drought, particularly in IC38-inoculated plants without or with 50%N, as well in IC55 + 50% N plants, as compared to control. In general, the highest content of soluble carbohydrate was registered in plants from inoculation treatments.

Drought-stressed plants growing without N displayed increase in cladode free aminoacids contents as compared to well-irrigated ones, whereas, in root, a similar response was registered in plants growing with 50% N (Fig. 8). Under drought, the highest root free aminoacids accumulation was observed in 50% N plants from uninoculated, IC38 and IC55 treatments compared to other N/inoculation treatments (Fig. 8).

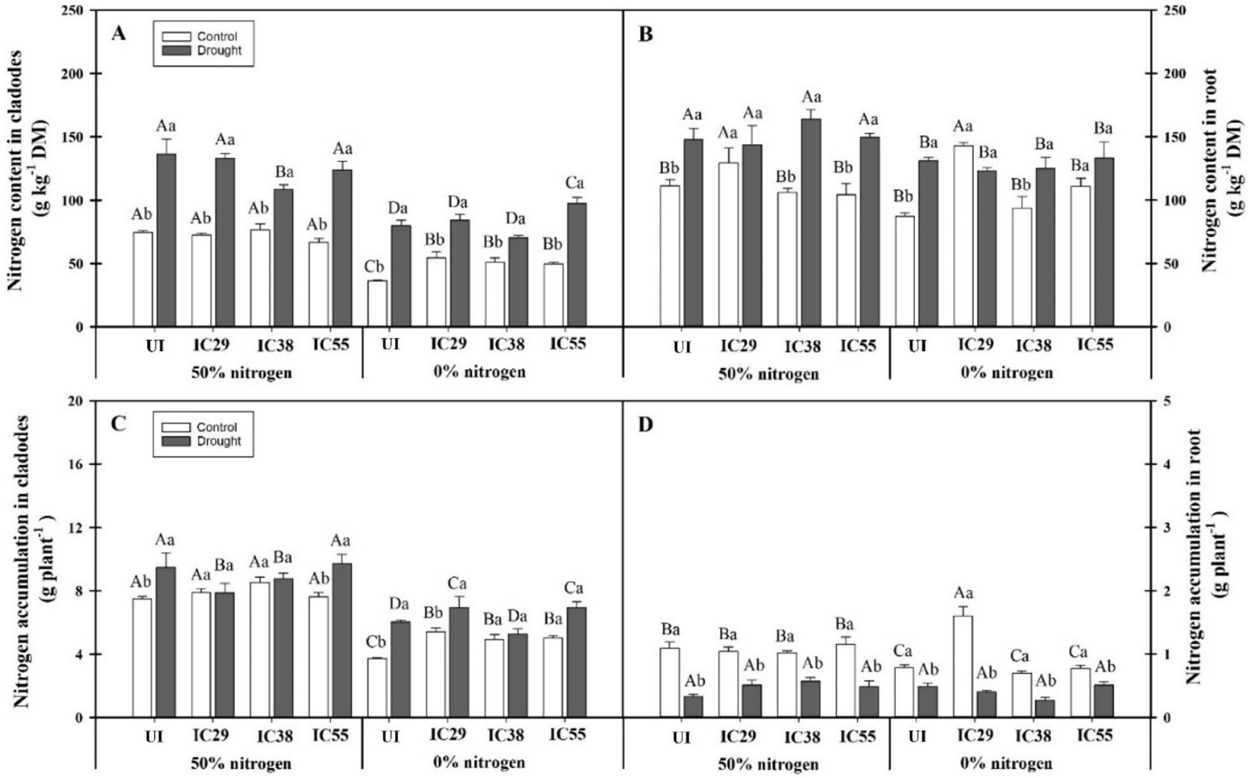


Fig. 6. Content (**A**, **B**) and accumulation (**C**, **D**) of nitrogen in cladodes and roots of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IC29) and *Paenibacillus* sp. (IC38 and IC55) strains, 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to drought stress (Control × Drought), using Scott-Knott test ($p < 0.05$).

Principal component analysis

To explore the main patterns of variation, a preliminary Principal Component Analysis (PCA) with all treatments was performed, identifying PC1 and PC2 as the most informative components with a cumulative variance of 82.07% and 90.90% for treatment without and with 50% N, respectively. For clarity, Fig. 9 presents a refined PCA to highlight a clear hierarchical clustering trend among the bacterial strains and drought stress.

Without N (Fig. 9A), the strains clustered distinctly in response to water regimes, forming four groups. Under well-irrigated conditions, the bacterial strains clustered (Group III - IC29, IC38 and IC55) distinctly from the uninoculated treatment (Group II). Conversely, under drought, the IC29 and IC55 strains (Group IV) were prominent, promoting higher nitrogen content in cactus pear. In contrast, the IC38 strain clustered with the uninoculated treatment (UI) (Group I), showing higher accumulation of free aminoacids, root soluble carbohydrates, chlorophyll *b*, and carotenoids.

Under 50% N (Fig. 9B), three groups were formed, but there was no separation between the strains (IC29, IC38 and IC55) and uninoculated (UI) (Group II) treatment in well-irrigated plants, which was strongly related to most biometric parameters, biomass, and water status. Conversely, under drought, a clustering occurred between the IC29 and IC38 strains (Group I), which was more correlated with nitrogen levels in the roots and cladodes, chlorophyll *a*, carotenoids, and root soluble carbohydrates. Group III was formed by the IC55 strain and UI treatment, which was correlated with chlorophyll *b*, total chlorophyll, cladode temperature, cladode nitrogen accumulation, and root free aminoacids.

The correlation network for each N and inoculation treatment reveals a strong positive correlation between biometric parameters, biomass and water status of the cactus pear plants, regardless of studied treatment (Fig. 10). However, the UI and the IC38 and IC55 strains treatments showed a significant negative correlation between carotenoids content with biometric and biomass parameters. Also, UI and IC55 strain showed a positive correlation between soluble carbohydrate and cladode succulence. Finally, the IC29 strain contributed to a higher nitrogen content in roots, which positively correlated with biomass and water status.

Discussion

Cactus pear resistance against drought can be improved by plant growth-promoting bacteria

In arid and semiarid regions, the soil fertility, high temperatures and limited water availability represent major constraints to agriculture. These limitations are particularly severe for fast-growing species, such as plants

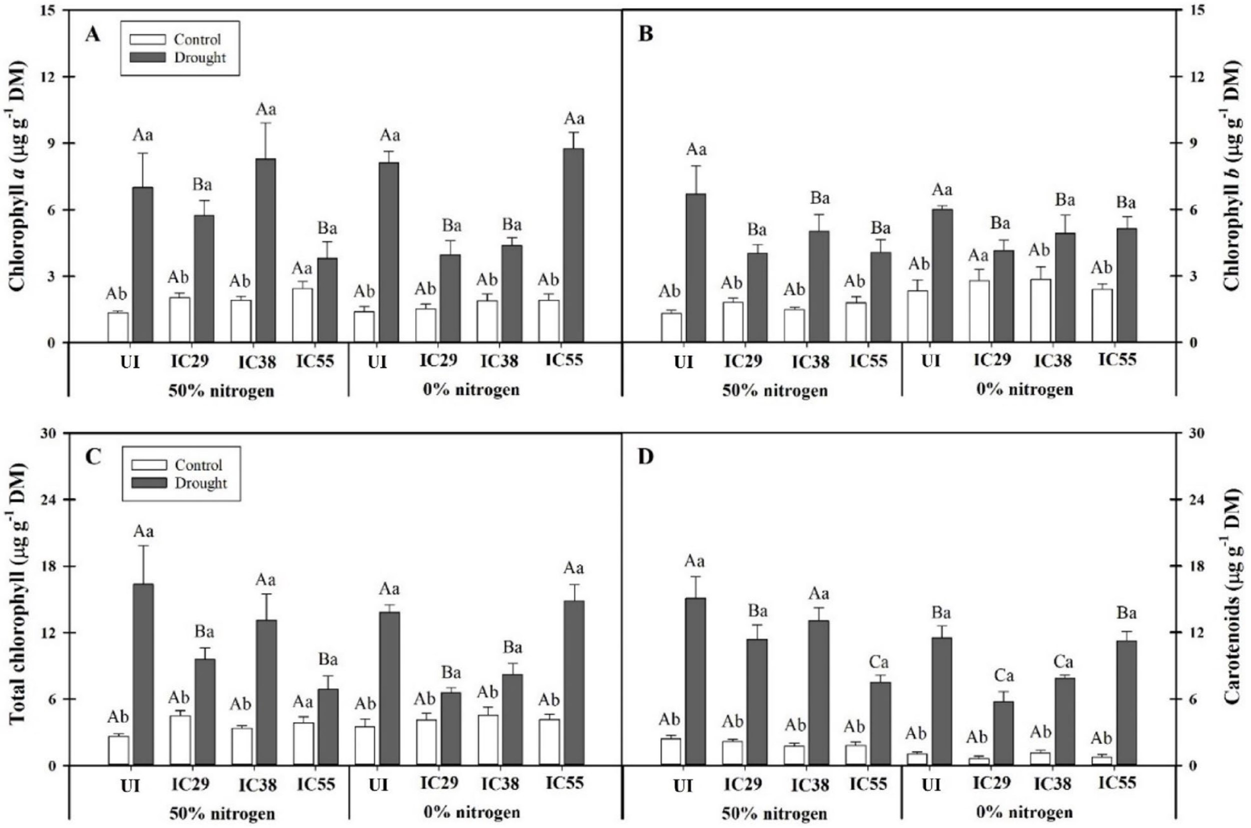


Fig. 7. Content of chlorophyll *a* (**A**), chlorophyll *b* (**B**), chlorophyll total (**C**) and carotenoids (**D**) of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IC29) and *Paenibacillus* sp. (IC38 and IC55) strains, 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to drought stress (Control × Drought), using Scott-Knott test ($p < 0.05$).

with C3 and C4 metabolism. In contrast, plants exhibiting CAM metabolism are promising alternatives for agricultural production, and developing sustainable and low-cost technologies plays a crucial role in promoting more resilient and productive agriculture[8,35,36]. In the current study, we investigate the role of selected plant growth-promoting bacteria strains, in association with nitrogen fertilization, on cactus pear performance under drought stress.

The best growth performance of cactus pear plants was registered under well-irrigated regime (50% FC) associated with 50% N in the growth medium (Figs. 2, 3 and 4). The data revealed that drought imposed severe limitations on the growth of forage cactus, with constraints on plant height, number of cladodes and biomass accumulation in both cladodes and roots (Fig. 2). These effects were more evident in plant morphology, where drought-stressed plants seemed to be less vigorous than well-irrigated ones, particularly in the development of the root system (Figs. 3 and 4).

The performance of forage cactus was differentially modulated by PGPB inoculation and N fertilization. The beneficial role of PGPB was more evident in plants under drought conditions, with the greatest benefits observed in stressed plants and in the absence of N. The data suggest a mutually beneficial interaction for plant growth, where IC29, IC38 and IC55 strains alleviate drought damage by providing fixed N under water deprivation to better root growth and drought tolerance (Figs. 2, 4 and 5C). In concordance, the PGPB strains were reported to induce mechanisms capable of enhancing plant growth under adverse conditions through biological nitrogen fixation or phytohormone production[16,18,37,41,45].

Superior performance under drought induced by plant growth-promoting bacteria is associated with metabolic adjustments

The water content into plant cells has been pointed as critical in drought-stressed plants to increase nitrogen uptake and its efficient use, promoting greater leaf expansion and, consequently, a larger light interception area[9,38]. This behavior was not found in our study, and the increase in cladode area (Fig. 2) most likely arises from other mechanisms, as drought imposes a similar decrease in cladode succulence and an increase in cladode temperature, without the influence of N/inoculation in these treatments (Fig. 5A, B). Another interesting result was that cactus pear plants activated intrinsic defense mechanisms against drought, including positive feedback for nitrogen accumulation in cladodes, which resulted in high contents of chlorophyll *a*, chlorophyll *b*, total

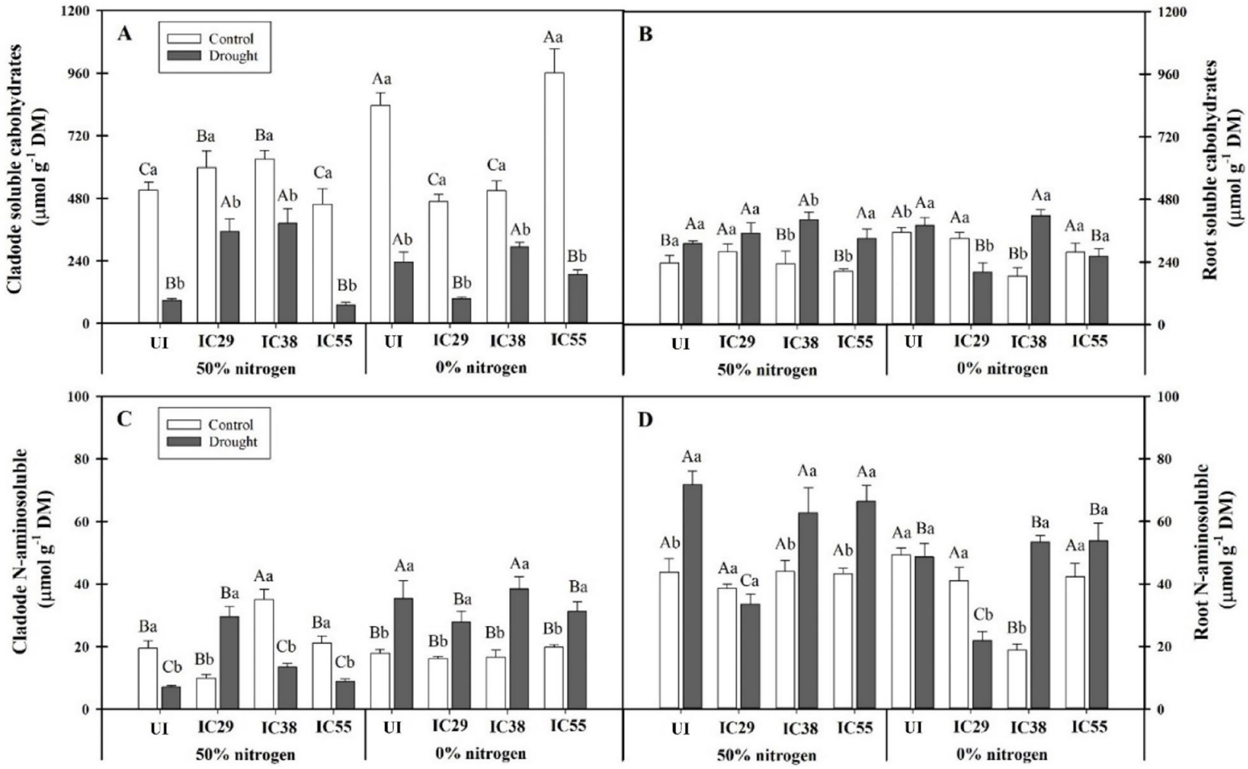


Fig. 8. Content of soluble carbohydrates (**A**, **B**) and free aminoacids (**C**, **D**) in cladode and roots of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IC29) and *Paenibacillus* sp. (IC38 and IC55) strains, 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to drought stress (Control × Drought), using Scott-Knott test ($p < 0.05$).

chlorophyll, and carotenoids (Figs. 6 and 7). This reprogramming appears to be a constitutive response of cactus pear plants, as there was no influence from the N/inoculation treatments. The accumulation of photosynthetic pigment under drought may reflect a response to maximize photosynthetic efficiency and protect tissues against oxidative damage[39]. Specifically, carotenoids play a protective role as antioxidants, helping to mitigate oxidative stress caused by prolonged exposure to water stress[40].

The data also evidence that superior drought tolerance of IC29-inoculated plants without N (Fig. 5C) was associated with root N accumulation (Figs. 6 and 10) and a fine tuning of photosynthetic pigments contents (Figs. 7 and 10), a different behavior of plants from other N/inoculation treatments (Fig. 9). The lower content of photosynthetic pigments in IC29-inoculated drought-stressed plants (Fig. 7) indicates a nitrogen economy under restricted conditions that can be utilized for other plant processes, including protein synthesis and plant growth. The results show that IC29 strain has an intrinsic ability to promote root growth, regardless of N exogenous. Previous studies have showed that IC29 strain may facilitate nutrient uptake or stimulating the production of phytohormones like auxins[41], as well act in acetylene reduction (a process catalyzed by nitrogenase from strain)[37], in order to promote the root elongation[42].

Multivariate assays may explain how PGPB modulate cactus pear performance under water restriction

Numerous reports highlight that PGPBs secrete extracellular polysaccharides (EPSs) in response to water stress[43,44]. These compounds may modify the root architecture and act as emulsifiers, mitigating the effects of reactive oxygen species (ROS). Moreover, plants inoculated with EPS-producing bacteria show a significant increase in accumulation of proline, carbohydrates and free amino acids, as well as in biomass growth, leaf area expansion, and protein content[44,45].

The accumulation of soluble carbohydrates is an important response under drought conditions, often associated with osmotic adjustment, as it helps maintain cell turgor and stabilize cellular structures under water-limited conditions. In this study, PCA and correlation network data indicate that the regulation of carbohydrate accumulation may vary depending on the source of nitrogen and PGPB inoculation (Figs. 8, 9 and 10). In the absence of inoculation or with strain IC55 inoculation, carbohydrate accumulation was directly related to cladode succulence, indicating its role in osmotic adjustment processes (Fig. 10). On the other hand, under IC29 and IC38 inoculation, carbohydrate synthesis was directly related to nitrogen accumulation, which in turn affects succulence and growth parameters (Fig. 10). These findings underscore the pivotal role of PGPB

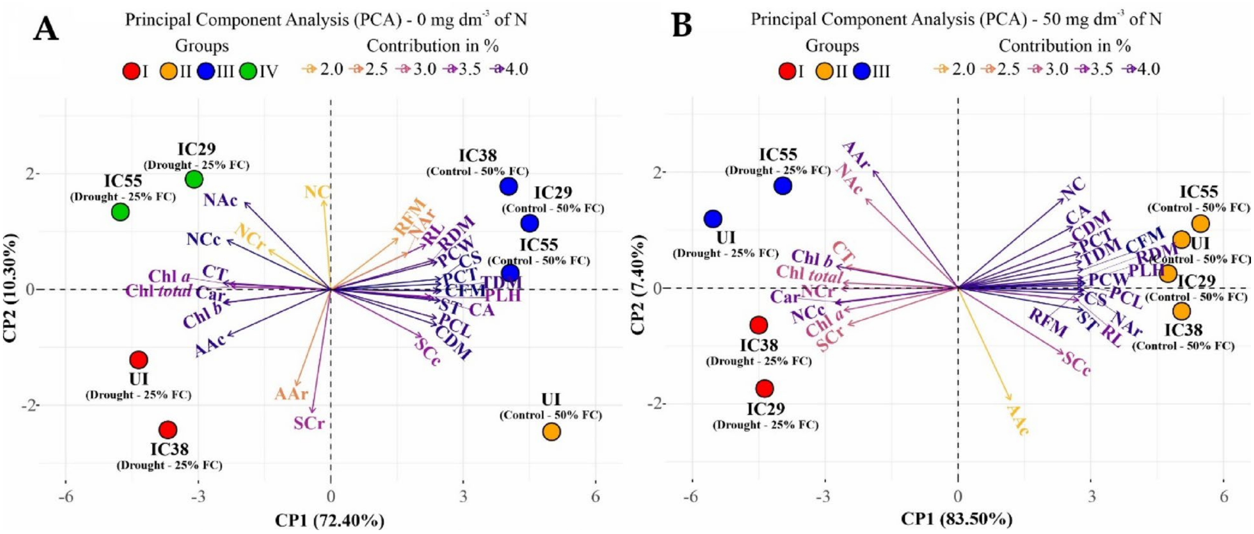


Fig. 9. Principal Component Analysis (PCA) for water regimes (control and drought) and treatments uninoculated (UI) or inoculation with *Bacillus subtilis* (IC29) and *Paenibacillus* sp. (IC38 and IC55) strains, without N (A) and with 50% N (B) in cactus pear plants. Variables: plant height (PLH), number of cladode (NC), cladode area (CA), root length (RL), primary cladode thickness (PCT), primary cladode length (PCL), primary cladode width (PCW), cladode fresh mass (CFM), clad dry mass (CDM), root fresh mass (RFM), root dry mass (RDM), total dry mass (TDM), cladode succulence (CS), stress tolerance (ST), cladode temperature (CT), chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl total), carotenoids (Car), N content in root (NCR), N accumulation in root (NAR), N content in cladode (NCC), N accumulation in cladode (NAC), root carbohydrates (RCa), cladode carbohydrates (CCa), root amino acids (RAA), cladode amino acids (CAA).

inoculation in modulating carbohydrate metabolism and promoting the accumulation of carbon-based energy reserves essential for stress resilience and development[46].

The results of free aminoacids reinforce the role of PGPB in osmoprotection and osmotic adjustment in cladode of drought-stressed plants without N (Fig. 8). The aminoacids synthesis pathway was closely related with uninoculated and IC38-inoculated stressed plants (Figs. 9 and 10), but the correlation network evidence that IC29-inoculated stressed plants exhibited a close relationship between aminoacids and photosynthetic pigments, suggesting a coordinated response involving nitrogen metabolism and photosynthetic efficiency. Taken together, these results indicate that strain IC29 exhibits the most promising performance among the tested PGPBs for inoculation in forage cactus under drought conditions. Future investigations must be performed to explain the specific role on PGPBs in nitrogen metabolism and drought tolerance mechanisms in CAM plants such as cactus pear.

Conclusion

The data reveal that PGPB alleviates the drought deleterious effects in cactus pear plants. The interaction between plant defense mechanisms and PGPB is particularly evident under N absence. The IPACC29 strain was the most promising for inoculation with forage cactus pear, activating important defense responses primarily related to nitrogen and organic compound accumulation, resulting in more developed roots and growth.

The strategic use of bacterial strains can contribute to crop resilience in adverse environments, reducing the need for fertilizers and increasing agricultural sustainability. The data indicate that the IPACC29, IPACC38 and IPACC55 strains can all be effective tools for improving the productivity of *Opuntia cochenillifera*, Miúda cultivar, in semiarid regions. This biotechnological management offers an alternative for cultivating cactus pear under water restriction and reduced N availability, providing a promising approach to enhance resistance and increasing green forage biomass production.

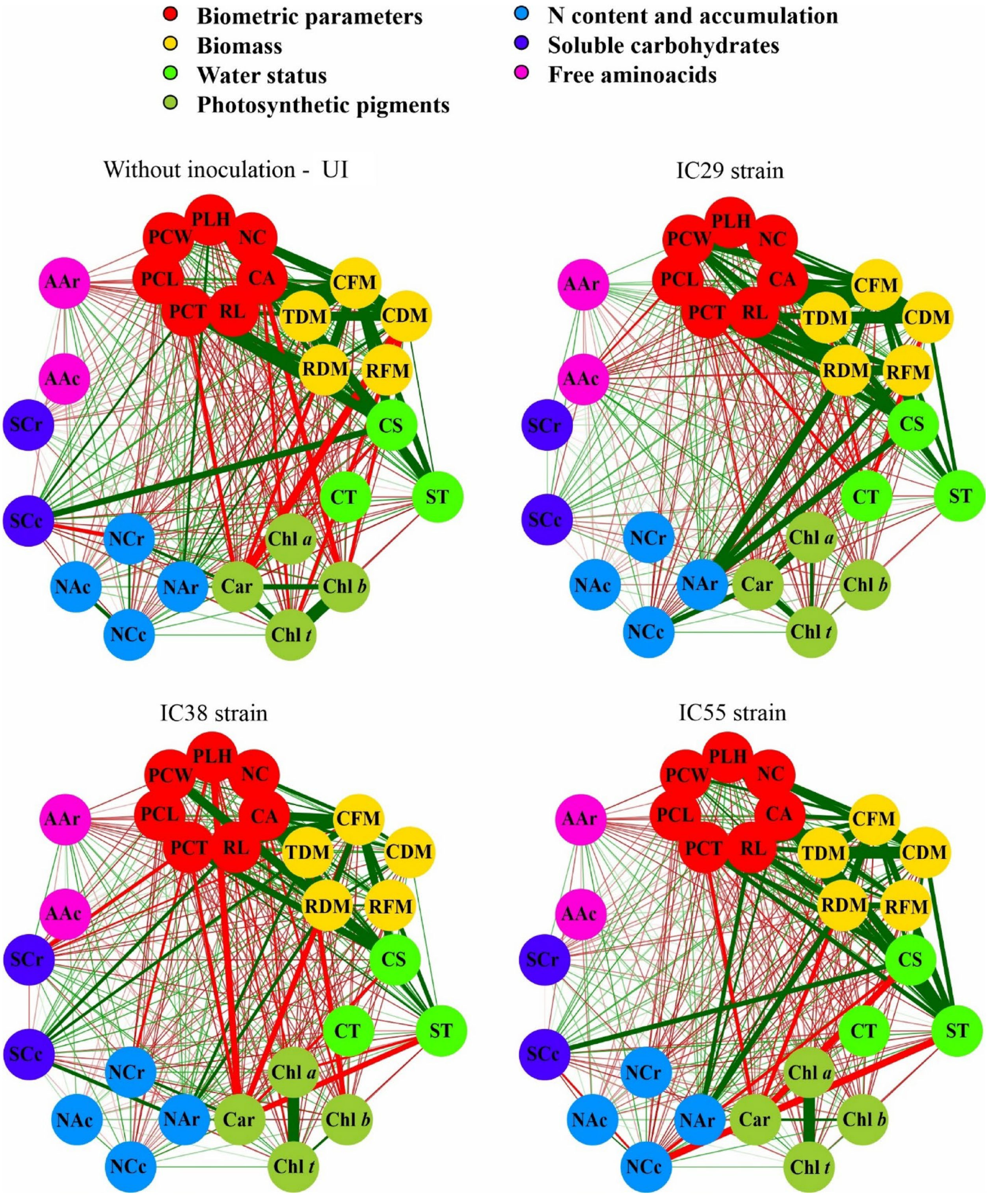


Fig. 10. Correlation network for bacterial strains and uninoculation (UI) treatments in cactus pear plants. The nodes represent individual variables grouped arbitrarily by similarity in nature, while the edges represent the relationships between variables (correlations). The edge thickness and color intensity correspond to magnitude and direction (positive or negative) of the correlation. Variables: plant height (PLH), number of cladode (NC), cladode area (CA), root length (RL), primary cladode thickness (PCT), primary cladode length (PCL), primary cladode width (PCW), cladode fresh mass (CFM), cladode dry mass (CDM), root fresh mass (RFM), root dry mass (RDM), total dry mass (TDM), cladode succulence (CS), stress tolerance (ST), cladode temperature (CT), chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl t), carotenoids (Car), N content in root (NCr), N accumulation in root (NAr), N content in cladode (NCc), N accumulation in cladode (NAc), root soluble carbohydrates (SCr), cladode soluble carbohydrates (SCc), root free aminoacids (AAr), and cladode free aminoacids (AAc).

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Received: 23 August 2024; Accepted: 15 September 2025

References

1. Fadiji, A. E., Santoyo, G., Yadav, A. N. & Babalola, O. O. Efforts towards overcoming drought stress in crops: revisiting the mechanisms employed by plant growth-promoting bacteria. *Front. Microbiol.* **13**, 962–427 (2022).
2. Singh, D. et al. Prospecting the potential of plant growth-promoting microorganisms for mitigating drought stress in crop plants. *Curr. Microbiol.* **81**, 81–84 (2024).
3. Soares, J. C., Santos, C. S., Carvalho, S. M. P., Pintado, M. M. & Vasconcelos, M. W. Preserving the nutritional quality of crop plants under a changing climate: importance and strategies. *Plant. Soil.* **443**, 1–26 (2019).
4. Ilyas, M. et al. Drought tolerance strategies in plants: a mechanistic approach. *J. Plant. Growth Regul.* **40**, 926–944 (2021).
5. Ali, S. & Khan, N. Delineation of mechanistic approaches employed by plant growth promoting microorganisms for improving drought stress tolerance in plants. *Microbiol. Res.* **249**, 126771 (2021).
6. Edvan, R. L. et al. Resilience of cactus Pear genotypes in a tropical semi-arid region subject to Climatic cultivation restriction. *Sci. Rep.* **10**, 10040 (2020).
7. Ramos, J. P. D. F. et al. Forage yield and morphological traits of cactus Pear genotypes. *Acta Sci. -Agron.* **43**, 51214 (2021).
8. Dubeux Jr, J. C. B. et al. Cactus (*Opuntia and Nopalea*) nutritive value: a review. *Anim. Feed Sci. Tech.* **275**, 114890 (2021).
9. Scalisi, A., Morandi, B., Inglese, P. & Bianco, R. L. Cladode growth dynamics in *Opuntia ficus*-indica under drought. *Environ. Exp. Bot.* **122**, 158–167 (2016).
10. Lahbouki, S. et al. Effects of humic substances and mycorrhizal fungi on drought-stressed cactus: focus on growth, physiology, and biochemistry. *Plants* **12**, 4156 (2023).
11. Edvan, R. L. et al. Potential use of hydrogel from natural fiber in the initial growth of *Nopalea Cochenillifera* under water stress: water source of slow release. *Commun. Soil. Sci. Plant.* **54**, 3063–3075 (2023).
12. Gupta, A. et al. Mechanistic insights of plant growth promoting bacteria mediated drought and salt stress tolerance in plants for sustainable agriculture. *Int. J. Mol. Sci.* **23**, 3741 (2022).
13. Salem, M. A., Ismail, M. A., Radwan, K. H. & Abd-Elhalim, H. M. Unlocking the potential of plant growth-promoting rhizobacteria to enhance drought tolerance in Egyptian wheat (*Triticum aestivum). Sustainability*. **16**, 4605 (2024).
14. Hungria, M., Campo, R. J., Souza, E. M. & Pedrosa, F. O. Inoculation with selected strains of *Azospirillum Brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant. Soil.* **331**, 413–425 (2010).
15. Yang, J., Martinson, T. E. & Liu, R. H. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chem.* **116**, 332–339 (2019).
16. Kavamura, V. N. et al. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. *Microbiol. Res.* **168**, 183–191 (2013).
17. Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M. & SkZ, A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiol. Res.* **184**, 13–24 (2016).
18. Danish, S., Zafar-ul-Hy, M., Mohsin, F. & Hussain, M. ACC-deaminase producing plant growth promoting rhizobacteria and Biochar mitigate adverse effects of drought stress on maize growth. *Plos One*. **15**, 230615 (2021).
19. Zhang, Q. & Rue, K. Effects of plant growth-promoting microorganisms on the early growth of Kentucky bluegrass under drought and salinity. *HortTechnology* **34**, 405–411 (2024).
20. Riseh, R. S., Ebrahimi-Zarandi, M., Vazvani, M. G. & Skorik, Y. A. Reducing drought stress in plants by encapsulating plant growth-promoting bacteria with polysaccharides. *Int. J. Mol. Sci.* **22**, 12979 (2021).
21. Antunes, J. E. L. et al. Diversity of plant growth promoting bacteria associated with sugarcane. *Genet. Mol. Res.* **16**, gmr16029662 (2017).
22. Aquino, J. P. A. et al. Plant growth-promoting bacteria increase the yield of green maize and sweet sorghum. *J. Plant. Nutr.* **46**, 58–68 (2022).
23. Sousa, A. B. Bactérias promotoras do crescimento de plantas e adubação nitrogenada no desenvolvimento de cultivares de palma forrageira em região semiárida [Master's thesis]. Federal University of Piauí, Teresina, Piauí, Brazil (2023).
24. Edvan, R. L. & Carneiro, M. S. S. *Palma forrageira: cultivo e uso na alimentação animal* 1st edn Brazil, 93 (Curitiba, 2019).
25. Sousa, D. M. G. & Lobato, E. *Cerrado: correção do solo e adubação* 2nd edn Brasil, 416 (Embrapa Informação Tecnológica, 2004).
26. Miranda, R. S. et al. Selection of soybean and cowpea cultivars with superior performance under drought using growth and biochemical aspects. *Plants*. **12**, 3134 (2023).
27. Mantovani, A. A method to improve leaf succulence quantification. *Braz Arch. Biol. Techn*. **42**, 9–14 (1999).
28. Wellburn, A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant. Physiol.* **144**, 307–313 (1994).
29. Baethgen, W. E. & Alley, M. M. A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. *Commun. Soil. Sci. Plant.* **20**, 961–969 (1989).
30. Fageria, N. K. & Baligar, V. C. Enhancing nitrogen use efficiency in crop plants. *Adv. Agron.* **88**, 97–185 (2005).
31. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T. & Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**, 350–356 (1956).
32. Yemm, E. W. & Cocking, E. C. The determination of amino acids with ninhydrin. *Analyst* **80**, 209–213 (1955).
33. Ferreira, D. F. SISVAR: A computer analysis system to fixed effects split plot type designs. *Rev. Bras. Biomet*. **37**, 529–535 (2019).
34. R Development Core Team. R: A language and environment for statistical computing; R foundation for statistical computing: Vienna, Austria Available online: (2016). http://www.R-project.org (accessed June 2024).
35. Lahbouki, S., Ech-chatir, L., Er-Raki, S., Outzourhit, A. & Meddich, A. Improving drought tolerance of *Opuntia ficus*-indica under field using subsurface water retention technology: changes in physiological and biochemical parameters. *Can. J. Soil. Sci.* **102**, 888–898 (2022).
36. Hossain, M. A., Hossain, M. S. & Akter, M. Challenges faced by plant growth-promoting bacteria in field-level applications and suggestions to overcome the barriers. *Physiol. Mol. Plant. P*. **126**, 102029 (2023).
37. Aquino, J. P. A. et al. Plant growth-promoting bacteria improve growth and nitrogen metabolism in maize and sorghum. *Theor. Exp. Plant. Phys.* **33**, 249–260 (2021).
38. Wang, H. et al. Optimization of water and fertilizer management improves yield, water, nitrogen, phosphorus and potassium uptake and use efficiency of cotton under drip fertigation. *Agr Water Manage.* **245**, 106662 (2021).
39. Abdallah, M. A., Methenni, K., Nouairi, I., Zarrouk, M. & Youssef, N. B. Drought priming improves subsequent more severe drought in a drought-sensitive cultivar of Olive cv. Chétoui. *Sci. Hortic.* **221**, 43–52 (2017).
40. Dhami, N. & Cazzonelli, C. I. Environmental impacts on carotenoid metabolism in leaves. *Plant. Growth Regul.* **92**, 455–477 (2020).

41. Chiappero, J., Cappellari, L. R., Alderete, L. G. S., Palermo, T. B. & Banchio, E. Plant growth promoting rhizobacteria improve the antioxidant status in *Mentha Piperita* grown under drought stress leading to an enhancement of plant growth and total phenolic content. *Ind. Crops Prod.* **139**, 111553 (2019).
42. Andrade, L. A., Santos, C. H. B., Frezarin, E. T., Sales, L. R. & Rigobelo, E. C. Plant growth-promoting rhizobacteria for sustainable agricultural production. *Microorganisms* **11**, 1088 (2023).
43. Ghosh, D., Gupta, A. & Mohapatra, S. A comparative analysis of exopolysaccharide and phytohormone secretions by four drought-tolerant rhizobacterial strains and their impact on osmotic-stress mitigation in *Arabidopsis Thaliana*. *World J. Microbiol. Biotechnol.* **35**, 90 (2019).
44. Chieb, M. & Gachomo, E. W. The role of plant growth promoting rhizobacteria in plant drought stress responses. *BMC Plant. Biol.* **23**, 407 (2023).
45. Khan, N. & Bano, A. Exopolysaccharide producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions. *Plos One*. **14**, e0222302 (2019).
46. Mohammadi, A. S. et al. Role of root hydraulics in plant drought tolerance. *J. Plant. Growth Regul.* **42**, 6228–6243 (2023).

Acknowledgements

The research was funded by the Foundation for Research Support of the State of Piauí (FAPEPI), under collaboration with Coordination for the Improvement of Higher Education Personnel (CAPES) grant number Edital nº 04/2021 - Apoio ao Desenvolvimento da Região Semiárida Brasileira, and by National Council for Scientific and Technological Development (CNPq), grant number 316056/2021-9.

Author contributions

Conceptualization, G.A.D., R.S.M. and E.M.C.; methodology, G.A.D., P.G.C.M., S.O.P.-M.; R.S.M. and E.M.C.; software, M.R.L.L., R.F.S., R.O.A.; validation, R.O.A., M.H.F.D, R.R.N. and R.L.E.; formal analysis, G.A.D., B.S.F.F., P.G.C.M., A.B.S., S.O.P.-M.; investigation, C.L.B., A.S.F.A., R.O.S. and J.H.C.; resources, R.S.M. and F.A.-N.; data curation, G.A.D., S.O.P.-M., M.R.L.L, and R.F.S.; writing-original draft preparation, R.S.M., G.A.D., S.O.P.-M., M.R.L.L, and R.F.S.; writing-review and editing, R.S.M., E.M.C. and A.S.F.A.; visualization, C.L.B., J.H.C. and F.A.-N.; supervision, R.S.M.; project administration, R.S.M. All authors have read and agreed to the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethics statement

The authors declare that this research was conducted ethically and responsibly, and all procedures were carried out according to relevant guidelines and regulations.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-20509-5.

Correspondence and requests for materials should be addressed to R.d.S.M.