



Article

Halotolerant Rhizobacteria Promote Plant Growth and Decrease Salt Stress in *Carya illinoensis* (Wangenh.) K. Koch

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Abstract: Pecan cultivation holds significant global importance. Salinity negatively affects the physiology and metabolism of the plant. However, certain plant growth-promoting rhizobacteria (PGPR) have the ability to reduce salt stress in plants. The objective was to analyze the effects of the rhizobacteria *Bacillus paralicheniformis* strain LBEndo1 on the development of pecan seedlings under salinity stress conditions. Two factors were analyzed: the presence of saline stress and the bacterial inoculation. The bacterial application was conducted at a concentration of 1×10^8 CFU/mL, and irrigation was administered every third day with 80 mL of water containing 50 mM NaCl. The results show that the rhizobacteria has a maximum tolerance of 15% salinity. Furthermore, the inoculation of PGPR significantly increased the biomass of the seedlings, including the dry weight of leaves, stem, and roots, as well as the stem diameter and height. Furthermore, seedlings that interacted with the rhizobacteria exhibited superior development under saline conditions, with significant increases of 105.5% in chlorophyll concentration and 112% in proline accumulation compared to non-inoculated seedlings. Additionally, a remarkable reduction in leaf damage caused by salt stress was observed. In conclusion, the LBEndo1 rhizobacteria, being a strain resistant to salinity and possessing multiple mechanisms to promote growth while mitigating salt stress, has the potential to be utilized in pecan seedlings to alleviate stress caused by salinity and increases biomass.

Keywords: PGPR; salinity stress; growth promotion; pecan



Citation: Palacio-Rodríguez, R.; Sáenz-Mata, J.; Trejo-Calzada, R.; Ochoa-García, P.P.; Arreola-Ávila, J.G. Halotolerant Rhizobacteria Promote Plant Growth and Decrease Salt Stress in *Carya illinoensis* (Wangenh.) K. Koch. *Agronomy* **2023**, *13*, 3045. <https://doi.org/10.3390/agronomy13123045>

Academic Editor: Fengjie Sun

Received: 7 November 2023

Revised: 3 December 2023

Accepted: 9 December 2023

Published: 13 December 2023



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1. Introduction

Carya illinoensis is a tree species native to the southern United States and northern Mexico, belonging to the Juglandaceae family, and is currently one of the most significant crops worldwide [1,2]. According to data from Sierra-Zurita et al. [3], Mexico ranks first in global pecan nut production, with an approximate cultivated area of 146,239 hectares and a production of 136,947 tons in 2021. In growing areas, including Mexico, nut seedlings from free pollination are used as rootstocks in established grafted orchards [4]. This plant thrives naturally in areas close to streams or rivers, with alluvial soils that are fertile, deep, well-drained, and possess excellent water retention capacity [5]. However, it is known for its sensitivity to salinity, with a tolerance range of approximately 2 to 3 dS·m⁻¹ of electrical conductivity. Values exceeding this range can cause a severe decrease in development, and the damage may even be fatal to the plant [6–8].

Salt-induced stress in the pecan tree results in reduced leaf production, stem diameter, and root development, leading to lower biomass and a negative impact on fruit production [9]. Additionally, high salt concentrations affect physiological characteristics such as stomatal conductance and photosynthetic capacity [10]. Furthermore, other metabolic aspects of plants can be affected, leading to osmotic stress and ionic imbalance, disrupting

water potential homeostasis [11]. Just as the overproduction of ethylene can trigger adverse effects such as accelerated senescence, it can also lead to the development of oxidative stress, impacting plant health [12].

The rhizosphere, defined as the soil zone influenced by the biological activity in the roots of plants, creates a conducive environment for symbiotic interaction between these roots and microorganisms. Numerous studies support the knowledge that beneficial bacteria in the rhizosphere can enhance biomass production and alleviate abiotic stress in plants [13]. These microorganisms are known as plant growth-promoting rhizobacteria (PGPR) [14,15]. Among their notable characteristics is the ability to activate defense mechanisms in plants, including the increased production of proline; this amino acid is crucial to enhance tolerance to abiotic stress, such as drought, nutrient imbalances, and high salinity concentrations. It acts as an osmoprotective agent, preserving cellular integrity and mitigating oxidative damage [16,17]. The use of PGPR has emerged as a promising approach to mitigate plant stress caused by high salt concentrations [18]. Ali et al. [19] demonstrated that the ACC deaminase enzyme produced by the bacterium *Pseudomonas fluorescens* can reduce salt stress in tomato plants by lowering the level of ethylene. Some benefits provided by rhizobacteria include increased fresh weight of plants, improved K^+/Na^+ ratio [20], enhanced germination, and larger plant size [21].

The inoculation of PGPR in soils with high electrical conductivity problems has been regarded as the most effective way to reduce the adverse effects of salinity on plants [22–24]. PGPR synthesize specific compounds that act as inhibitors of signaling cascades in response to salt stress [25]. Additionally, they exert direct mechanisms involving biological nitrogen fixation, phytohormone production, phosphate solubilization, toxicity reduction, all contributing to enhanced water and nutrient absorption. Some bacteria reported with this capacity have been the genus *Bacillus* [26,27].

Despite the global importance of this crop, the plant's susceptibility to high salt concentrations, and the relevance of PGPR in agriculture, few studies have addressed this issue. Building on previous research demonstrating the promoting effect of *B. paralicheniformis* LBEndo1 in *Arabidopsis thaliana*, we decided to expand our analysis to determine if these benefits manifest similarly in pecan seedlings. Therefore, this current study was conducted with the aim of examining the effects of rhizobacteria on the development of pecan seedlings, especially under saline stress conditions. Additionally, we sought to evaluate the rhizobacteria's ability to tolerate saline stress. Given the nature of the experiments, it was crucial for the rhizobacteria to demonstrate resistance to high salt concentrations.

2. Materials and Methods

2.1. Plant Material

The experiments were conducted at the facilities of the Faculty of Biological Sciences, Juárez University of the State of Durango (25°35'13" N and 103°30'04" W, at an altitude of 1114 m above sea level). For the experiments, seeds from native pecan trees were collected from an orchard located in the municipality of Nazas, Durango (25°15'08" N and 104°06'09" W, at an altitude of 1240 m above sea level). The seeds were disinfected with 20% sodium hypochlorite, then rinsed with sterile distilled water. Subsequently, they were cold stratified at 4 °C for one week before being germinated in sterile sand and irrigated with sterile water every third day. After the pecan seedlings germinated, they were transplanted into pots measuring 20 cm in height and 10 cm in diameter. The substrate used was sterilized sand. Throughout the entire experiment, the seedlings were irrigated only with sterile distilled water, without introducing any additional nutrient solutions. The experiment was conducted in a bioclimatic chamber under controlled temperature conditions (25 °C) and photoperiods (16 h of light and 8 h of darkness).

2.2. Experimental Design

For this experiment, a completely randomized 2×2 factorial design (four treatments) with three replications was established, with each replication consisting of five seedlings

(15 seedlings per treatment), resulting in a total of 60 experimental units. The first variable factor was the plant growth-promoting rhizobacteria *B. paralicheniformis* LBEndo1 at two levels (presence and absence), and the second factor was the concentration of NaCl (0 and 50 mM). Irrigations were performed every third day, applying 80 mL of saline solution or without salts (salt-free control) over a period of 60 days. The codes for the four treatments were designated as follows: Seedlings without bacteria or salt = Control, seedlings with bacteria without salt = LBEndo1, seedlings without bacteria with salt = Control+S, and seedlings with bacteria with salt = LBEndo1+S.

2.3. Determination of Halotolerance in the Rhizobacteria LBEndo1

A series of experiments were carried out with the objective of determining the tolerance of the bacterial strain LBEndo1 to saline stress. To achieve this, the liquid Luria Bertani (LB) culture medium was used as a base and enriched with different concentrations of NaCl (5%, 10%, and 15% *w/v*) to simulate saline stress conditions. The rhizobacteria were cultured and subsequently incubated at a temperature of 35 °C, with constant agitation at 120 rpm to provide optimal growth conditions. After a three-day period, an evaluation of bacterial growth was conducted. A count was performed using a Neubauer chamber to determine the number of colony-forming units per milliliter (CFU/mL) and thereby confirm bacterial growth in the different saline concentrations.

2.4. Bacterial Inoculation

To carry out this analysis, the rhizobacterial strain *B. paralicheniformis* LBEndo1 was utilized. For its cultivation, 100 mL of LB liquid medium were added to an Erlenmeyer flask, which was then incubated for 24 h at a constant temperature of 35 °C, with continuous agitation at 120 rpm. After the cultivation period, the bacterial concentration was adjusted to 1×10^8 CFU/mL using a Neubauer chamber. Before inoculation into the seedlings, the bacteria were centrifuged at 5000 rpm for 5 min to remove the culture medium; they were then resuspended in sterilized distilled water. The seedlings used in the assay were one week old from germination, and the inoculation was performed at the base of the stem by applying 10 mL of the bacteria previously adjusted to 1×10^8 CFU/mL.

2.5. Biostimulation of *C. illinoensis* by the Rhizobacteria LBEndo1

After a 60-day period of interaction between rhizobacteria, seedlings, and salinity, morphometric measurements were conducted to analyze the growth of all seedlings. Using an analytical balance, the dry weight of leaves, stems, and roots was determined, while stem diameter was measured with a caliper. Additionally, the height of the 15 seedlings from each treatment (60 seedlings in total) was recorded weekly to observe the growth dynamics during the 60 days of the experiment. Upon conclusion, the obtained results allowed for the evaluation of the effects of rhizobacteria on seedling growth.

2.6. Extraction and Measurement of Proline

The extraction and quantification of proline were performed following the methodology of Bates et al. [28]. For this analysis, samples of fresh tissue (leaves) were taken from the seedlings of each of the treatments. Using an analytical balance, 100 mg of the aerial part of *C. illinoensis* seedlings were weighed, and the plant material was placed in an Eppendorf tube containing a 140 mM sulfosalicylic acid solution to be ground with a pestle. The resulting solution was filtered through a 0.20 µm filter. The liquid extract was mixed with a solution of 140 mM acidic ninhydrin and glacial acetic acid in a 1:1:1 ratio. The tubes were heated to 100 °C for one hour, then cooled on ice. Toluene was added in a 1:1 ratio, vigorously vortexed, and allowed to settle. The organic fraction was extracted and placed in a quartz cuvette for measuring the absorbance of the ninhydrin-proline complex at 520 nm using spectrophotometry. A calibration curve was previously established using concentrations of 10, 20, 40, 50, 75, and 100 µg of commercial proline (Sigma Aldrich Chemical Co., Burlington, MA, USA®).

2.7. Extraction and Measurement of Chlorophyll

The assay for chlorophyll measurement was conducted on the leaves of seedlings from each of the treatments, following the protocol of Zhang and Huang [29]. The total chlorophyll was extracted from 0.1 g of fresh *C. illinoensis* leaves immersed in Eppendorf tubes with N, N-dimethylformamide (DMF) for 24 h at a temperature of 4 °C in darkness. The concentrations of chlorophyll A and chlorophyll B were measured by spectrophotometry at absorbance wavelengths of 664 nm and 647 nm, respectively. The content of chlorophyll A, B, and total chlorophyll (A + B) was determined by substituting the obtained absorbances into the following formulas:

$$\text{Chlorophyll: A} = 12.7 \times A_{664} - 2.79 \times A_{647}$$

$$\text{Chlorophyll: B} = 20.7 \times A_{647} - 4.62 \times A_{664}$$

$$\text{Chlorophyll: A + B} = 17.90 \times A_{647} + 8.08 \times A_{664}$$

2.8. Statistical Analysis

A statistical analysis was performed to evaluate the different developmental parameters in this study. This included the analysis of root, stem, and leaf dry weights, stem diameter, seedling height, and proline and chlorophyll levels. The experimental design was set up in a completely randomized manner. For the statistical analysis, both the Student's *t*-test and ANOVA method were used, followed by a Tukey post hoc test for multiple comparisons. The level of significance was set at $p \leq 0.05$. All statistical analyses were conducted using GraphPad Prism 9 software.

3. Results

3.1. Halotolerance of the Rhizobacteria LBEndo1

One of the keys features a rhizobacteria must possess to be used in reducing salt stress in seedlings is the ability to tolerate high salinity concentrations [14]. After three days of bacterial strain growth in LB liquid medium enriched with NaCl (0, 5, 10, and 15%), it was determined that the maximum salt tolerance capacity of the bacteria was 15% *w/v*. However, it is evident that at a higher salt concentration, bacterial growth decreases (Figure 1).

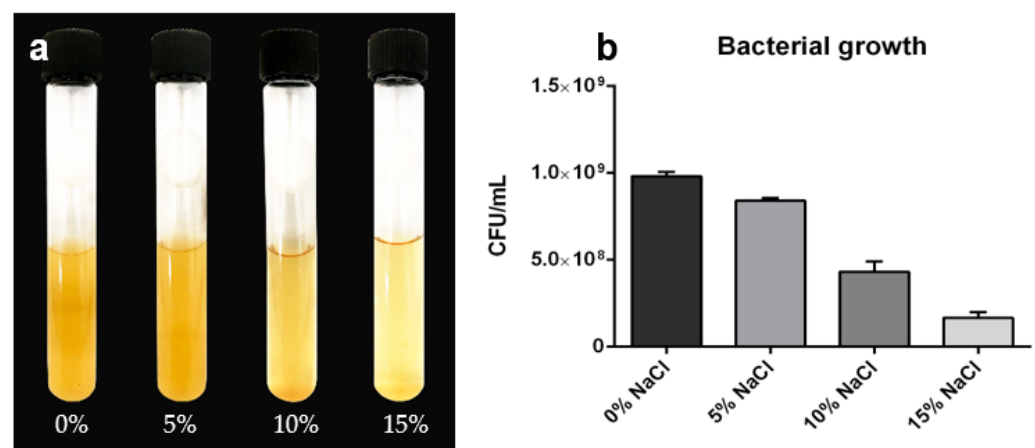


Figure 1. Tolerance of bacteria to salinity (a) Growth of NaCl-tolerant rhizobacteria in tubes with LB medium supplemented with different salt concentrations (0%, 5%, 10%, and 15%); (b) Graph illustrating the quantity of CFU/mL of the bacteria grown in various NaCl concentrations.

3.2. Seedling Growth Dynamics

During the experiment, it was found that the seedlings in the treatment inoculated with *B. paralicheniformis* LBEndo1, but without exposure to salt stress (LBEndo1), exhibited significantly greater growth compared to the other conditions. On the other hand, the seedlings that were not in contact with the rhizobacteria showed a lower stem height, with this difference being more pronounced in the Control+S treatment. This suggests that salinity has a negative impact on seedling development, while rhizobacteria beneficially contribute to the growth of seedlings, even under saline conditions. It was also observed that both the LBEndo1+S treatment and the Control+S treatment displayed slower growth in the last three weeks of the experiment compared to the LBEndo1 and Control treatments (Figure 2). These results support the importance of rhizobacteria inoculation in promoting optimal seedling growth, especially under salt stress conditions.

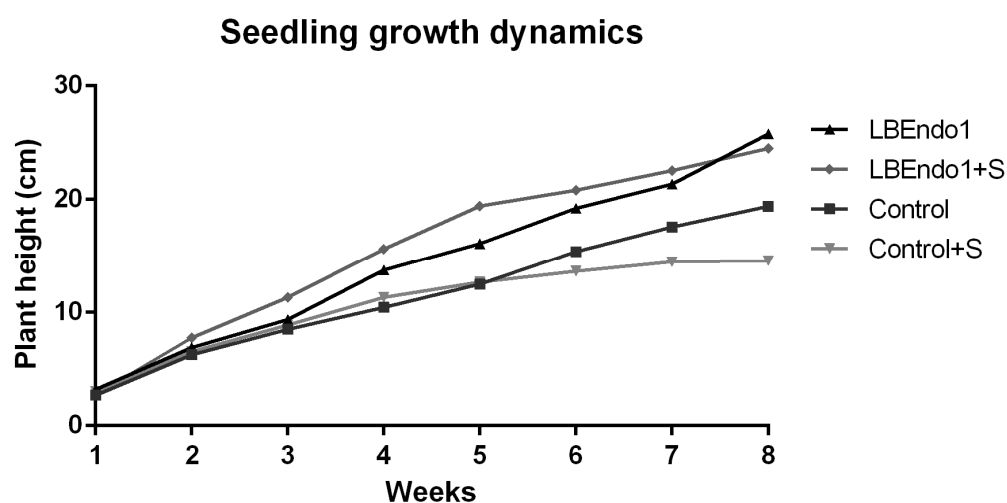


Figure 2. Height changes were recorded in pecan seedlings subjected to four different treatments: Control, LBEndo1, Control+S, and LBEndo1+S. The measurements were carried out over a 60-day period, with weekly measurements taken over the course of eight weeks.

3.3. Growth Promotion under Saline and Non-Saline Stress Conditions

The results obtained under non-saline stress conditions show that the inoculated seedlings exhibited higher biomass production (Figure 3). In each of the morphometric parameters evaluated, seedlings interacting with the rhizobacteria (LBEndo1) demonstrated superior performance compared to the control treatment (Control), as evidenced in Table 1. Specifically, the growth-promoting effect was most pronounced in the dry weight of the leaves, showing an average increase of 81.5%. This result indicates greater accumulation of leaf biomass in the inoculated seedlings. Additionally, dry weights of roots and stems also showed significant increases, with respective increments of 59.3% and 58.9%. These findings suggest improved development of the root system and structural tissue. Regarding seedling height, an average increase of 32.8% was observed in seedlings interacting with the bacteria. It is noteworthy that, although there was a 9.63% increase in stem diameter in the inoculated seedlings, this difference was not statistically significant compared to the Control treatment. This suggests that LBEndo1 bacteria may have a limited effect on stem thickening, although a slight increasing trend was still observed.

On the other hand, under saline stress conditions, positive changes were observed in various morphometric parameters in seedlings inoculated with rhizobacteria (LBEndo1+S) compared to the control group (Control+S), detailed in Table 1. Firstly, a significant increase in stem dry weight was achieved, with an average increase of 98.7%. Likewise, for the leaves, a 163.3% increase in dry weight was recorded, demonstrating the positive effect of rhizobacteria on foliar growth under salinity conditions. Regarding the roots, an average increase of 112.9% in dry weight was observed in the presence of rhizobacteria. In relation to

seedling height, despite saline stress, an average increase of 68.3% was achieved in seedlings with rhizobacteria. Furthermore, a 34.2% increase in stem diameter was recorded, indicating increased resistance and structural rigidity in seedlings treated with rhizobacteria. These findings demonstrate that the rhizobacterium *B. paralicheniformis* LBEndo1 has the ability to increase the biomass of *C. illinoensis* seedlings even under saline stress conditions. Despite both plants experiencing damage due to salinity, it is evident that the inoculated seedlings exhibited less harm than the non-bacterially inoculated ones, suggesting the positive influence of rhizobacteria in alleviating saline stress in seedlings (Figure 3).



Figure 3. Photographs depicting the appearance of *C. illinoensis* seedlings from the Control, LBEndo1, Control+S, and LBEndo1+S treatments at 60 days post-inoculation. These images illustrate both the growth-promoting effect induced by rhizobacteria and the damage caused by saline stress.

Table 1. Morphometric data of the Control, LBEndo1, Control+S, and LBEndo1+S treatments. Different letters indicate significant differences between treatment means according to ANOVA analysis ($p \leq 0.05$).

	Leaf Dry Weight (g)	Stem Dry Weight (g)	Root Dry Weight (g)	Total Dry Weight (g)	Seedling Height (cm)	Stem Diameter (cm)
Control	1.3 ± 0.19 c	1.6 ± 0.19 c	10.1 ± 0.94 b	13.0 ± 0.89 b	19.3 ± 0.67 b	3.7 ± 0.39 a
LBEndo1	2.4 ± 0.13 a	2.5 ± 0.10 a	16.0 ± 2.02 a	21.0 ± 2.18 a	25.7 ± 1.22 a	4.1 ± 0.34 a
Control+S	0.7 ± 0.13 d	1.1 ± 0.10 d	6.9 ± 0.65 c	8.7 ± 0.67 c	14.5 ± 1.37 c	2.9 ± 0.35 b
LBEndo1+S	1.9 ± 0.22 b	2.2 ± 0.16 b	14.6 ± 1.06 a	18.9 ± 0.75 a	24.4 ± 1.37 a	3.9 ± 0.23 a

Data are shown as mean ± standard deviation (SD, n = 60).

3.4. Proline Content

To assess the impact of the LBEndo1 rhizobacteria on proline accumulation, measurements were carried out in seedlings inoculated with these rhizobacteria and in the non-inoculated control group, both under saline stress and non-saline conditions. In the case of leaves without saline stress, seedlings inoculated with the rhizobacteria showed a 54.2% increase in proline production, reaching a level of 31 $\mu\text{mol g}^{-1}$, in contrast to control seedlings that produced 20.1 $\mu\text{mol g}^{-1}$. On the other hand, in seedlings under saline stress, the control group recorded a production of 34.5 $\mu\text{mol g}^{-1}$, while in interaction with the rhizobacteria they achieved a production of 56.6 $\mu\text{mol g}^{-1}$, representing a 64.05% increase.

It is important to note that the Control+S and LBEndo1 treatments showed a similar proline production (Figure 4a).

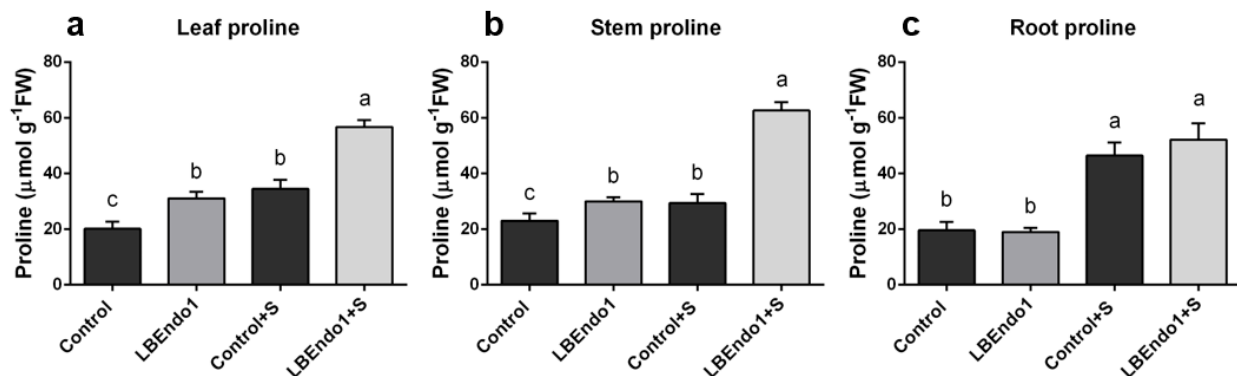


Figure 4. Proline production in the leaves (a), stem (b), and root (c) of *C. illinoensis* after 60 days of interaction. Values represent mean $\pm$ standard deviation ($n = 60$). Different letters indicate significant differences between treatment means according to ANOVA analysis ($p \leq 0.05$).

Regarding the stems, under non-saline stress conditions, the Control treatment had an average of $23 \mu\text{mol g}^{-1}$, 30% less than seedlings treated with LBEndo1, which produced $29.9 \mu\text{mol g}^{-1}$. In the case of seedlings exposed to saline stress, the Control+S and LBEndo1+S treatments produced $29.4 \mu\text{mol g}^{-1}$ and $62.6 \mu\text{mol g}^{-1}$, respectively, with the inoculated treatment showing a 112.9% higher production (Figure 4b). Once again, a similar proline production was observed between the Control+S and LBEndo1 treatments.

As for the roots of seedlings under non-saline conditions, a statistically similar proline production was observed, with an accumulation of $19.5 \mu\text{mol g}^{-1}$ in the Control treatment and $18.9 \mu\text{mol g}^{-1}$ in seedlings inoculated with rhizobacteria, in this case, slightly below the Control treatment by 3.07%. Similarly, in the roots of seedlings subjected to saline stress, no significant differences were found between the Control+S and LBEndo1+S treatments, with a production of $46.4 \mu\text{mol g}^{-1}$ and $52.1 \mu\text{mol g}^{-1}$, respectively (Figure 4c), representing a 12.2% increase compared to the Control treatment. These results indicate that seedlings inoculated with LBEndo1 show a higher proline accumulation, even in the absence of stress conditions.

3.5. Chlorophyll Content and Foliar Damage

Analysis of total chlorophyll measurement was conducted on 60-day-old *C. illinoensis* leaves. Under saline stress conditions, the LBEndo1+S treatment exhibited a 105.5% increase in chlorophyll concentration compared to the Control+S treatment. LBEndo1-inoculated seedlings displayed chlorophyll values of $14.4 \mu\text{g mL}^{-1}$, while the control group reached only $7.01 \mu\text{g mL}^{-1}$. This finding suggests that bacterial inoculation with LBEndo1 had a positive effect on chlorophyll production in *C. illinoensis* seedlings under saline stress (Figure 5a).

Conversely, under non-saline stress conditions, a similar trend was observed, where the LBEndo1 treatment had a total chlorophyll concentration of $20.23 \mu\text{g mL}^{-1}$, while the Control treatment only reached $10.08 \mu\text{g mL}^{-1}$. The difference between the two treatments was 100.6%, indicating that bacterial inoculation also favored chlorophyll production under non-saline stress conditions (Figure 5a). These results are promising as bacterial inoculation with LBEndo1 can enhance chlorophyll concentration in *C. illinoensis* seedlings. This pigment is crucial for photosynthesis and seedling growth, thus an increase in its concentration could have a positive impact on the seedlings' ability to cope with adverse conditions.

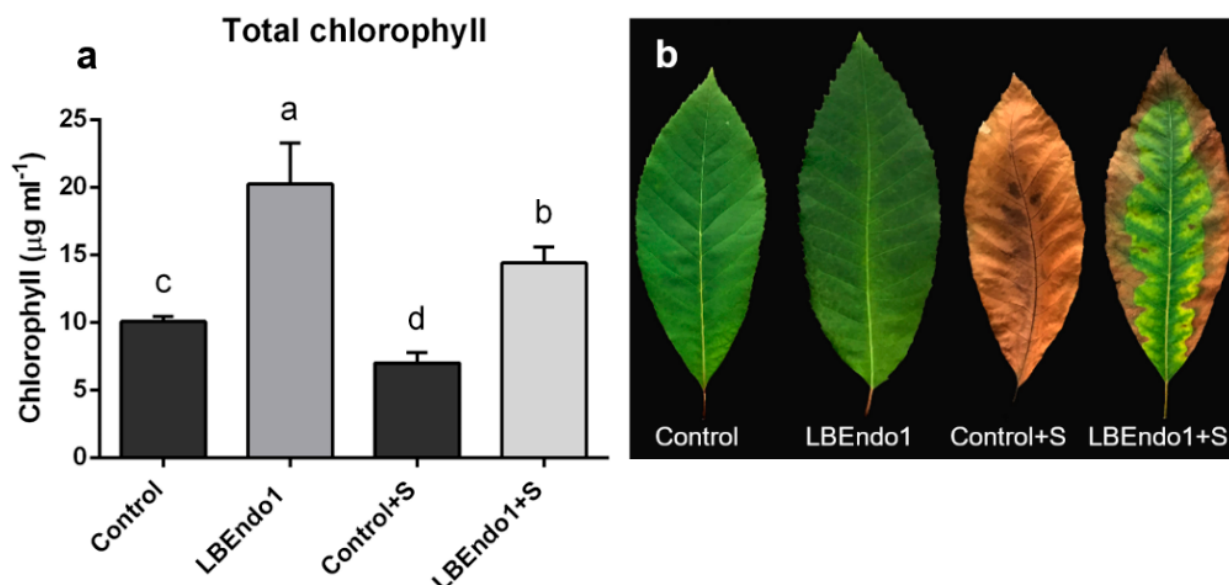


Figure 5. Effect of LBEndo1 rhizobacteria on total chlorophyll production in *C. illinoensis* leaves under saline and non-saline stress conditions. **(a)** Total chlorophyll in each of the four treatments. Different letters indicate significant differences between treatment means according to ANOVA analysis ($p \leq 0.05$) compared to the respective control. **(b)** Photograph illustrating the effect of NaCl on leaves and the reduction of damage in the LBEndo1+S treatment.

Additionally, leaf damage assessments were conducted to analyze the impact of saline stress. Treatments that were not subjected to salt irrigation exhibited healthy-looking leaves. Conversely, wilting was observed in the leaves of treatments exposed to NaCl. However, a delayed effect was observed in seedlings inoculated with the LBEndo1 bacteria. Notably, the Control+S treatment was more severely affected, showing 100% leaf damage, while the LBEndo1+S treatment exhibited lower damage at 42.5% (Figure 5b). Therefore, it can be inferred that the LBEndo1 rhizobacteria could provide some protection against the adverse effects of saline stress on seedling leaves.

4. Discussion

The results of this research demonstrate the beneficial capacity of the rhizobacterium *B. paralicheniformis* LBEndo1 to stimulate growth and improve salt stress tolerance in *C. illinoensis* seedlings. These findings confirm the initial hypothesis that proposed that inoculation with this rhizobacterium could provide advantages to seedlings under salinity conditions, suggesting a relationship between the presence of the rhizobacterium and the vitality and performance of seedlings under salt stress.

Our findings align with previous research, such as that of Zhang et al. [30] and Jiao et al. [31], indicating the susceptibility of *C. illinoensis* to salinity. The negative response of this species to salinity is in line with the current scientific understanding. These results underscore the importance of addressing salt stress in managing the productivity and quality of pecan nuts.

Similar to the findings of Etesami and Beattie [32], our results indicate that the *B. paralicheniformis* strain LBEndo1 is resistant to high saline concentrations (Figure 1) and has the ability to promote plant growth, as well as alleviate salt stress; in their work, they investigated the growth-promoting effect mediated by bacteria of the *Bacillus* genus. They discovered that *Bacillus* strains with salt tolerance demonstrate a greater ability to confer benefits to plants under salt stress conditions [33,34]. The description of bacterial species from the genus *Bacillus* as plant growth promoters has also been reported by Ibarra-Villarreal [35]. The rhizobacterium *B. paralicheniformis* has been acknowledged as a PGPR

with the ability to enhance the biomass of various agriculturally relevant plants, as well as to alleviate the adverse effects of different types of abiotic stresses [36,37].

Research conducted by Khalilpour et al. [38] has indicated that plant growth-promoting bacteria can influence plant growth by enhancing nutrient uptake, mineral solubilization, and phytohormone production. In their study, they observed an increase in the growth of *Pistacia vera* following inoculation with different plant growth-promoting bacteria, as also reported by Salimi et al. [39]. Similarly, our findings reveal a significant increase in the growth of seedlings inoculated with *B. paralicheniformis* LBEndo1 in the absence of salt stress (Table 1), and these results are supported by previous research highlighting the rhizobacterium's ability to stimulate pecan seedling development. These observations also align with studies conducted by Shi et al. [40], who have demonstrated that plant–microorganism interactions can have a positive impact on the growth of these plants.

The results indicating a greater accumulation of leaf biomass and an increase in root and stem dry weight in seedlings interacting with the rhizobacterium *B. paralicheniformis* LBEndo1 under salt stress conditions (Figure 4) align with research that has highlighted the positive influence of plant growth-promoting bacteria on plant development and architecture. These findings also relate to studies that have investigated the mechanisms that could be involved in enhancing plant growth. In their study, Shabaan et al. [41] examined the effect of *Acinetobacter johnsonii* inoculation on maize growth under salt stress. They found that bacteria-inoculated plants showed a significant increase in leaf biomass accumulation and an increase in root and stem dry weight compared to non-inoculated plants. This result supports the notion that plant growth-promoting bacteria can have a positive impact on plant development under salt stress conditions. Similarly, research such as that conducted by Pérez-Jaramillo et al. [42] has also explored how bacteria can influence root architecture and enhance nutrient uptake. In their study, they found that certain plant growth-promoting bacteria can modify the branching pattern of roots, resulting in a greater capacity to explore the soil for nutrients.

Proline plays a crucial role as a compatible osmolyte, serving its vital function in maintaining osmotic balance and cellular homeostasis under stress situations, thus preventing dehydration and loss of cell turgor [43]. A study by Ji et al. [44] reinforced the relationship between *Bacillus subtilis* inoculation and proline accumulation in plants. In their research, they explored the effects of plant growth-promoting bacteria on the response of wheat plants to salt stress. They observed that bacteria-inoculated plants exhibited a significant increase in proline accumulation compared to non-inoculated plants. This suggests that the bacteria might be actively inducing proline accumulation in the plant's stress response. Additionally, research such as that of Ashraf and Foolad [45] has emphasized the importance of proline accumulation in the adaptive response of plants to osmotic stress derived from salt stress. These studies align with the results obtained in our research, where a significant increase in proline production was evident in seedlings inoculated with *B. paralicheniformis* LBEndo1, both under salt stress conditions and in stress-free environments (Figure 4). These results indicate that inoculation with this rhizobacterium not only promotes greater proline accumulation but also imparts greater tolerance to salt stress in plants, as has been extensively reported by Shahid et al. [46].

In our project, a significant increase in chlorophyll concentration in the leaves of seedlings inoculated with the rhizobacterium *B. paralicheniformis* LBEndo1 is revealed, both under salt stress conditions and stress-free conditions (Figure 5a). This observation suggests that the rhizobacterium could be exerting a positive impact on seedling photosynthetic efficiency and metabolism. These results are similar to the studies by Kamran et al. [47], where it has been demonstrated that plant growth-promoting bacteria can enhance chlorophyll concentration in plants subjected to salt stress. Furthermore, research such as that of Han et al. [48] has supported the association between bacterial inoculation and increased chlorophyll concentration, implying a direct influence on plant photosynthesis and performance. Collectively, these findings support the idea that the rhizobacterium *B. paralicheniformis* LBEndo1 might be optimizing the chlorophyll concentration in seedling leaves. The in-

crease in chlorophyll concentration in the leaves of seedlings inoculated with the LBEndo1 rhizobacteria could be related to the previously described findings of increased proline accumulation. This coincidence in the results suggests a possible relationship between the increase in chlorophyll and proline in plants under saline stress, as also reported by Altuntaş et al. [49], supporting the idea that salt stress tolerance enhancing mechanisms can have multiple beneficial effects on plant physiology.

The protective effect observed in the leaves of seedlings inoculated with the LBEndo1 rhizobacterium against damage induced by salt stress (Figure 5b) underscores its ability to mitigate the adverse effects of salinity on plant health. For instance, research conducted by Khan et al. [50] has demonstrated that plant growth-promoting bacteria can induce the production of antioxidant enzymes in plants, counteracting the oxidative stress caused by salinity. On the other hand, a study by Munns and Tester [51] examined the impact of salt stress on plant physiology and highlighted how high salt concentrations in the soil can negatively affect the function of cell membranes and photosynthetic processes, resulting in evident leaf damage. In this context, the decrease in the percentage of leaf damage between the Control+S and LBEndo1+S treatments suggests that the rhizobacterium might be enhancing seedling resistance to salt stress, possibly through the induction of antioxidant defense mechanisms or other biochemical processes.

5. Conclusions

The bacterium *B. paralicheniformis* LBEndo1 exhibited a remarkable capacity for salt stress tolerance, reaching a maximum of 15% NaCl. However, a decrease in bacterial growth was observed as the salt concentration increased.

The application of the LBEndo1 rhizobacterium had a positive impact on pecan seedling growth, both under saline stress conditions and normal conditions. Inoculated seedlings showed a notable increase in dry biomass production, with enhancements in dry leaf, stem, and root weights compared to non-inoculated seedlings.

Furthermore, the bacterium was found to influence the accumulation of proline, a key indicator of stress resistance. Seedlings inoculated with the bacterium exhibited an increase in proline production under both saline and non-saline conditions, suggesting the bacterium's influence on the seedlings' response to salt stress.

Regarding chlorophyll production, a bacterium-induced essential increase was observed in pecan seedlings. This elevation in chlorophyll concentration is crucial for photosynthesis and, consequently, for seedling growth and development.

The lack of research focused on addressing salt stress in pecan seedlings through PGPR renders our study particularly relevant. To better understand how LBEndo1 alleviates salt stress in pecan trees, further research of a metabolic and molecular nature is needed. These additional studies could not only support our findings but also bolster the use of PGPR in agriculture. This study underscores the need to strategically incorporate PGPR to create more efficient and salt-resistant agricultural systems.

Author Contributions: R.P.-R.: conceptualization, methodology, data analysis, writing and original draft preparation, and editing; J.S.-M., R.T.-C., and J.G.A.-A.: review and resources; P.P.O.-G.: conceptualization and data analysis. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: All data will be made available on request to the corresponding author's email with appropriate justification.

Acknowledgments: Rubén Palacio Rodríguez expresses gratitude for the financial support provided by the National Council of Science and Technology of Mexico (CONACYT) for his doctoral studies, as well as the support and sponsorship of the project by the Arid Zones Regional University Unit at the Autonomous University of Chapingo. We also wish to acknowledge the Faculty of Biological Sciences at UJED for providing the necessary strains for this study and for offering the infrastructure that facilitated the experiment's development.

Conflicts of Interest: The authors declare that they have no competing interests.

References

1. Rani, S.; Sharma, A.; Wali, V.K.; Kour, K.; Sharma, M.; Sharma, M. Evaluation of genetic diversity of pecan nut [*Carya illinoensis* (Wang) K. Koch.] in Jammu region. *Indian J. Hortic.* **2017**, *74*, 601–603. [\[CrossRef\]](#)
2. Oliveira de Oliveira, L.; Carlos Beise, D.; Damian Dos Santos, D.; Caroline Nagel, J.; Poletto, T.; Poletto, I.; Stefenon, V.M. Molecular markers in *Carya illinoensis* (Juglandaceae): From genetic characterization to molecular breeding. *J. Hortic. Sci. Biotechnol.* **2021**, *96*, 560–569. [\[CrossRef\]](#)
3. Sierra-Zurita, D.; Santana-Espinoza, S.; Rosales-Serna, R.; Ríos-Saucedo, J.C.; Carrillo-Parra, A. Productivity and Characterization of Biomass Obtained from Pruning of Walnut Orchards in México. *Energies* **2023**, *16*, 2243. [\[CrossRef\]](#)
4. Casales, F.G.; van der Watt, E.; Coetzer, G.M. Propagation of Pecan (*Carya illinoensis*): A review. *Afr. J. Biotechnol.* **2018**, *17*, 586–605. [\[CrossRef\]](#)
5. Wolstenholme, B.N. The ecology of pecan trees. 2. Climatic aspects of growing pecans. *Pecan Q.* **1979**, *13*, 14–19.
6. Miyamoto, S.; Riley, T.; Gobran, G.; Petticrew, J. Effects of saline water irrigation on soil salinity, pecan tree growth and nut production. *Irrig. Sci.* **1986**, *7*, 83–95. [\[CrossRef\]](#)
7. Miyamoto, S. *Supplement of Diagnosis and Management of Salinity Problems in Irrigated Pecan Production: Salt Leaching*; Technical Report N° 387A; Texas Water Resources Institute: College Station, TX, USA, 2010; 12p, Available online: <https://core.ac.uk/download/pdf/147136462.pdf> (accessed on 6 December 2023).
8. Miyamoto, S.; Nesbitt, M. Effectiveness of soil salinity management practices in basin-irrigated pecan orchards. *HortTechnology* **2011**, *21*, 569–576. [\[CrossRef\]](#)
9. Moreno-Izaguirre, E.; Ojeda-Barrios, D.; Avila-Quezada, G.; Guerrero-Prieto, V.; Parra-Quezada, R.; Ruiz-Anchondo, T. Sodium sulfate exposure slows growth of native pecan seedlings. *Phyton* **2015**, *84*, 80. [\[CrossRef\]](#)
10. Campos-Villarreal, A.G.; Arreola-Ávila, J.G.; Chávez-Simental, J.A.; Calzada, R.T.; de la Rosa, A.B.; Santos, A.L.; Salgado, J.R.H. Respuesta fisiológica, acumulación iónica y peso seco en portainjertos de nogal pecanero (*Carya illinoensis* (Wangenh) k. Koch) desarrollados bajo condiciones de estrés salino. *Interciencia* **2017**, *42*, 744–749.
11. Shahid, M.A.; Sarkhosh, A.; Khan, N.; Balal, R.M.; Ali, S.; Rossi, L.; Gómez, C.; Mattson, N.; Nasim, W.; Garcia-Sanchez, F. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy* **2020**, *10*, 938. [\[CrossRef\]](#)
12. Zhu, J.K. Genetic analysis of plant salt tolerance using Arabidopsis. *Plant Physiol.* **2000**, *124*, 941–948. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Angelina, E.; Papatheodorou, E.M.; Demirtzoglou, T.; Monokrousos, N. Effects of *Bacillus subtilis* and *Pseudomonas fluorescens* Inoculation on Attributes of the Lettuce (*Lactuca sativa* L.) Soil Rhizosphere Microbial Community: The Role of the Management System. *Agronomy* **2020**, *10*, 1428. [\[CrossRef\]](#)
14. Bhattacharyya, P.N.; Jha, D.K. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1327–1350. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Goswami, M.; Suresh, D.E.K.A. Plant growth-promoting rhizobacteria alleviators of abiotic stresses in soil: A review. *Pedosphere* **2020**, *30*, 40–61. [\[CrossRef\]](#)
16. Dobbelaere, S.; Vanderleyden, J.; Okon, Y. Plant growth-promoting effects of diazotrophs in the rhizosphere. *Crit. Rev. Plant Sci.* **2003**, *22*, 107–149. [\[CrossRef\]](#)
17. Mattioli, R.; Marchese, D.; D'Angeli, S.; Altamura, M.M.; Costantino, P.; Trovato, M. Modulation of intracellular proline levels affects flowering time and inflorescence architecture in Arabidopsis. *Plant Mol. Biol.* **2008**, *66*, 277–288. [\[CrossRef\]](#)
18. Fu, Q.; Liu, C.; Ding, N.; Lin, Y.; Guo, B. Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium *Pseudomonas* sp. DW1 on growth of eggplant (*Solanum melongena* L.) seedlings under salt stress. *Agric. Water Manag.* **2020**, *97*, 1994–2000. [\[CrossRef\]](#)
19. Ali, S.; Charles, T.C.; Glick, B.R. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. *Plant Physiol. Biochem.* **2014**, *80*, 160–167. [\[CrossRef\]](#)
20. Shilev, S.; Sancho, E.D.; Benlloch-González, M. Rhizospheric bacteria alleviate salt-produced stress in sunflower. *J. Environ. Manag.* **2012**, *95*, S37–S41. [\[CrossRef\]](#)
21. Yao, L.; Wu, Z.; Zheng, Y.; Kaleem, I.; Li, C. Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. *Eur. J. Soil Biol.* **2010**, *46*, 49–54. [\[CrossRef\]](#)
22. Glick, B.R.; Cheng, Z.; Czarny, J.; Duan, J. Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur. J. Plant Pathol.* **2007**, *119*, 329–339. [\[CrossRef\]](#)
23. Mokrani, S.; Nabti, E.H.; Cruz, C. Current advances in plant growth promoting bacteria alleviating salt stress for sustainable agriculture. *Appl. Sci.* **2020**, *10*, 7025. [\[CrossRef\]](#)
24. Moon, Y.S.; Khan, M.; Khan, M.A.; Ali, S. Ameliorative symbiosis of *Serratia fonticola* (S1T1) under salt stress condition enhance growth-promoting attributes of *Cucumis sativus* L. *Symbiosis* **2023**, *89*, 283–297. [\[CrossRef\]](#)
25. Nadeem, S.M.; Zahir, Z.A.; Naveed, M.; Ashraf, M. Microbial ACC-deaminase: Prospects and applications for inducing salt tolerance in plants. *Crit. Rev. Plant Sci.* **2010**, *29*, 360–393. [\[CrossRef\]](#)

26. Mohamed, H.I.; Gomaa, E.Z. Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescens* on growth and pigment composition of radish plants (*Raphanus sativus*) under NaCl stress. *Photosynthetica* **2012**, *50*, 263–272. [\[CrossRef\]](#)
27. Ha-Tran, D.M.; Nguyen, T.T.M.; Hung, S.H.; Huang, E.; Huang, C.C. Roles of plant growth-promoting rhizobacteria (PGPR) in stimulating salinity stress defense in plants: A review. *Int. J. Mol. Sci.* **2021**, *22*, 3154. [\[CrossRef\]](#)
28. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [\[CrossRef\]](#)
29. Zhang, Z.; Huang, R. Analysis of malondialdehyde, chlorophyll proline, soluble sugar, and glutathione content in *Arabidopsis* seedling. *Bio-Protocol* **2013**, *3*, 817. [\[CrossRef\]](#)
30. Zhang, J.; Jiao, Y.; Sharma, A.; Shen, D.; Wei, B.; Hong, C.; Zheng, B.; Pan, C. Transcriptomic analysis reveals potential pathways associated with salt resistance in pecan (*Carya illinoensis* K. Koch). *J. Biotechnol.* **2021**, *330*, 17–26. [\[CrossRef\]](#)
31. Jiao, Y.; Zhang, J.; Pan, C. Genome-wide analysis of the GDSL genes in pecan (*Carya illinoensis* k. koch): Phylogeny, structure, promoter cis-elements, co-expression networks, and response to salt stresses. *Genes* **2022**, *13*, 1103. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Etesami, H.; Beattie, G.A. Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Front. Microbiol.* **2018**, *9*, 148. [\[CrossRef\]](#)
33. Paul, D.; Lade, H. Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: A review. *Agron. Sustain. Dev.* **2020**, *34*, 737–752. [\[CrossRef\]](#)
34. Patani, A.; Prajapati, D.; Ali, D.; Kalasariya, H.; Yadav, V.K.; Tank, J.; Bagatharia, S.; Joshi, M.; Patel, A. Evaluation of the growth-inducing efficacy of various *Bacillus* species on the salt-stressed tomato (*Lycopersicon esculentum* Mill.). *Front. Plant Sci.* **2023**, *14*, 1168155. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Ibarra-Villarreal, A.L.; Gándara-Ledezma, A.; Godoy-Flores, A.D.; Herrera-Sepúlveda, A.; Díaz-Rodríguez, A.M.; Parra-Cota, F.I.; de los Santos-Villalobos, S. Salt-tolerant *Bacillus* species as a promising strategy to mitigate the salinity stress in wheat (*Triticum turgidum* subsp. durum). *J. Arid Environ.* **2021**, *186*, 104399. [\[CrossRef\]](#)
36. Du, Y.; Ma, J.; Yin, Z.; Liu, K.; Yao, G.; Xu, W.; Fan, L.; Du, B.; Ding, Y.; Wang, C. Comparative genomic analysis of *Bacillus paralicheniformis* MDJK30 with its closely related species reveals an evolutionary relationship between *B. paralicheniformis* and *B. licheniformis*. *BMC Genom.* **2019**, *20*, 283. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Liu, J.; Fimognari, L.; de Almeida, J.; Jensen, C.N.G.; Compant, S.; Oliveira, T.; Baelum, J.; Pastar, M.; Sessitsch, A.; Moelbak, L.; et al. Effect of *Bacillus paralicheniformis* on soybean (*Glycine max*) roots colonization, nutrient uptake and water use efficiency under drought stress. *J. Agron. Crop Sci.* **2023**, *209*, 547–565. [\[CrossRef\]](#)
38. Khalilpour, M.; Mozafari, V.; Abbaszadeh-Dahaji, P. Tolerance to salinity and drought stresses in pistachio (*Pistacia vera* L.) seedlings inoculated with indigenous stress-tolerant PGPR isolates. *Sci. Hortic.* **2021**, *289*, 110440. [\[CrossRef\]](#)
39. Salimi, F.; Khorshidi, M.; Amirahmadi, F.; Amirahmadi, A. Effectiveness of Phosphate and Zinc Solubilizing *Paenarthrobacter nitroguajacolicus* P1 as Halotolerant Rhizobacterium with Growth-Promoting Activity on *Pistacia vera* L. *Curr. Microbiol.* **2023**, *80*, 336. [\[CrossRef\]](#)
40. Shi, J.W.; Lu, L.X.; Shi, H.M.; Ye, J.R. Effects of plant growth promoting rhizobacteria on the growth and soil microbial community of *Carya illinoensis*. *Curr. Microbiol.* **2022**, *79*, 352. [\[CrossRef\]](#)
41. Shabaan, M.; Asghar, H.N.; Zahir, Z.A.; Zhang, X.; Sardar, M.F.; Li, H. Salt-tolerant PGPR confer salt tolerance to maize through enhanced soil biological health, enzymatic activities, nutrient uptake and antioxidant defense. *Front. Microbiol.* **2022**, *13*, 901865. [\[CrossRef\]](#)
42. Pérez-Jaramillo, J.E.; Carrión, V.J.; Bosse, M.; Ferrão, L.F.; De Hollander, M.; Garcia, A.A.; Ramírez, C.A.; Mendes, R.; Raaijmakers, J.M. Linking rhizosphere microbiome composition of wild and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits. *ISME J.* **2017**, *11*, 2244–2257. [\[CrossRef\]](#)
43. Hosseini, M.; Stefaniak, S.; Ghorbani Javid, M.; Soltani, E.; Wojtyla, L.; Garnczarska, M. Contribution of exogenous proline to abiotic stresses tolerance in plants: A review. *Int. J. Mol. Sci.* **2022**, *23*, 5186. [\[CrossRef\]](#)
44. Ji, C.; Tian, H.; Wang, X.; Song, X.; Ju, R.; Li, H.; Liu, X. *Bacillus subtilis* HG-15, a halotolerant rhizoplane bacterium, promotes growth and salinity tolerance in wheat (*Triticum aestivum*). *BioMed Res. Int.* **2022**, *2022*, 9506227. [\[CrossRef\]](#)
45. Ashraf, M.F.M.R.; Foolad, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **2007**, *59*, 206–216. [\[CrossRef\]](#)
46. Shahid, S.; Shahbaz, M.; Maqsood, M.F.; Farhat, F.; Zulfiqar, U.; Javed, T.; Fraz Ali, M.; Alhomrani, M.; Alamri, A.S. Proline-induced modifications in morpho-physiological, biochemical and yield attributes of pea (*Pisum sativum* L.) cultivars under salt stress. *Sustainability* **2022**, *14*, 13579. [\[CrossRef\]](#)
47. Kamran, M.; Imran, Q.M.; Ahmed, M.B.; Falak, N.; Khatoon, A.; Yun, B.W. Endophyte-mediated stress tolerance in plants: A sustainable strategy to enhance resilience and assist crop improvement. *Cells* **2022**, *11*, 3292. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Han, L.; Zhang, M.; Du, L.; Zhang, L.; Li, B. Effects of *Bacillus amyloliquefaciens* QST713 on photosynthesis and antioxidant characteristics of Alfalfa (*Medicago sativa* L.) under drought stress. *Agronomy* **2022**, *12*, 2177. [\[CrossRef\]](#)
49. Altuntaş, C.; Demiralay, M.; Sezgin Muslu, A.; Terzi, R. Proline-stimulated signaling primarily targets the chlorophyll degradation pathway and photosynthesis associated processes to cope with short-term water deficit in maize. *Photosynth. Res.* **2020**, *144*, 35–48. [\[CrossRef\]](#) [\[PubMed\]](#)

50. Khan, M.A.; Asaf, S.; Khan, A.L.; Adhikari, A.; Jan, R.; Ali, S.; Imran, M.; Kim, K.-M.; Lee, I.J. Halotolerant rhizobacterial strains mitigate the adverse effects of NaCl stress in soybean seedlings. *BioMed Res. Int.* **2019**, *2019*, 9530963. [[CrossRef](#)] [[PubMed](#)]
51. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [[CrossRef](#)] [[PubMed](#)]

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