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Bl: idea, design, conducting experiments, and writing of manuscript; RD and HARY: analyzing results; AD: conducting microbiological techniques

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## ORIGINAL RESEARCH PAPER

# Role of some rhizospheric *Pseudomonas* on the growth and physiology of broad bean (*Vicia faba*) under salt stress conditions

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**Abstract**

Salt stress affects the development and growth of plants in various ways as a result of its effect on water relationships, photosynthesis, and nutrient absorption by physiological and biochemical processes. Consequently, several researchers have increasingly studied the effect of plant growth promoting bacteria (PGPR) as promoters and enhancers under saline environment. The main goals of this study were to examine the manifested response of the broad bean plant under saline conditions and to evaluate the role of some *Pseudomonas* isolates in improving plant tolerance to salt stress. Three *Pseudomonas* strains were isolated (P1 and P7 from a saline soil and P15 from a vineyard soil). These isolates were screened by salinity and used as inoculums in *Vicia faba* plants (OTONO variety) irrigated with two saline solutions (NaCl; 100 and 150 mM L<sup>-1</sup>) and one without salinity. The results show that salinity decreased the fresh weight, total chlorophyll content, and the Na<sup>+</sup>/K<sup>+</sup> ratio, but it increased proline accumulation in inoculated and noninoculated plants. The inoculation of *V. faba* plants with P1, P7, and P15 strains significantly increased the production of fresh biomass in the presence and absence of salt stress, and positively affected the accumulation of proline and the Na<sup>+</sup>/K<sup>+</sup> ratio. The inoculation with bacterial strains increased the total chlorophyll content in plants at all salt treatment levels, especially the P1 strain that showed a significant effect.

**Keywords**

salinity; PGPR; proline; chlorophyll

**Introduction**

Salinity is considered to be one of the main abiotic stresses in the Sahara region because it reduces the area of exploitable land by 1% to 2% yearly [1]. Over 800 million hectares of land are affected by salinity, which could drastically reduce agricultural productivity [2]. In addition, salinity reduces nutrient absorption by plants, particularly phosphate uptake [3]. Osmotic stress can also be induced by limiting water absorption in soil, and ionic stress resulting from high concentrations of potentially toxic salt ions in plant cells [4]. Saline stress affects several biochemical and metabolic processes in plants, including protein synthesis, photosynthesis, and lipid metabolism, therefore, growth and yields are reduced [5,6]. The accumulation of ions, such as sodium chloride, can alter many physiological activities [7], decrease productivity, and cause plant death [2].

Agricultural soils face great risks due to their excessive and irrational exploitation; salinity is among these risks, which represents a serious constraint for agriculture. Against these risks of phytotoxicity of salinity, many researchers have studied several strategies, such as the use of plant growth-promoting bacteria (PGPR or PGPB). PGPRs

can stimulate not only plant growth and yield, but also alleviate the effects of biotic or abiotic stresses [8]. They can also increase plant growth and aerial biomass, even under the toxic effect of metals [9–11], and facilitate the growth of plants in saline soils [4,12]. The increase in crop yields due to PGPRs is mainly attributed to the production of growth phytohormones as well as the solubilization of phosphate [13]. Several authors have reported the enhancing effect of plant–PGPR interactions using different bioinoculant bacterial strains, such as *Azospirillum*, *Agrobacterium*, *Pseudomonas*, and several strains of gram-positive *Bacillus* [14,15]. Inoculation with *P. putida* Rs198 may stimulate cotton growth and germination under salt stress conditions [16]. The strains *P. trivialis* 3Re27 and *P. extremorientalis* TSAU20 have excellent root colonization capacity and promote plant growth. They also show antagonism to fungal plant pathogens, tolerance to salinity, and ability to alleviate salt stress in peas, soybeans, wheat, cucumber, and tomato [17–19]. In the present study, the effect of salt stress on biomass, chlorophyll content, proline accumulation, and absorption of nutritional elements was examined. We also studied the effect of inoculation with selected *Pseudomonas* strains on the growth of plants and their tolerance to salt stress.

## Material and methods

### Soil sampling

Ten soil samples were taken from two types of potentially contaminated soil: saline soil from Metmar Relizan and vineyard soil from Wreiah Mostaganem, Algeria.

### Isolation of *Pseudomonas* spp. strains

The isolation was carried out by the suspension dilution method described by Vidhyasekaran et al. [20]. The roots were first removed from the easily detachable soil, then 1 g of soil adhering to the roots of each sample was recovered and placed in a series of 1/10 dilution suspensions. Finally, the bacteria belonging to the genus *Pseudomonas* were isolated from the greenish-yellow fluorescent colonies after 48-h incubation [21] on King B medium, as described by King et al. [22]. Confirmation of the fluorescence of *Pseudomonas* strains was done either by naked eye or by using a UV lamp (366 nm), and therefore occurred after macroscopic and microscopic analyses.

### Selection and identification of high-performance isolates

The strains of fluorescent *Pseudomonas* passed through three preselection tests of performance: tolerance to salinity, and indole acetic acid (IAA) and pyoverdine production. Every isolate was incubated for 48 h in King B medium with different salinity levels: 7%, 8%, 9%, and 10% NaCl. Then, the best performing isolates were examined for their ability to produce pyoverdine. We used the method described by Meyer and Abdallah [23] for the extraction and spectrophotometric characterization of pyoverdine, and the method described by Loper and Schroth [24] for characterizing IAA production in these strains. The selected isolates were identified using the biochemical method of the API 20NE gallery.

### Experiment of plantation

The experiment was carried out in a greenhouse at the Agronomy Workshop in Mazragran, Abdelhamid Ibn Badis University in Mostaganem, Algeria ( $x$ : 35°53'05.79" N,  $y$ : 0°02'41.54" E) with an average temperature of 28°C by day and 23°C by night, and hygrosopy of 55% to 75%.

We used the seeds of *Vicia faba* OTONO. The seeds were germinated after disinfection with a 25% sodium hypochlorite solution for 15 min, and then transplanted into

pots containing 5 kg of mineralized and sterile sand. From colonies incubated for 24 h we prepared the inoculums of three strains selected for inoculation in tubes containing 3 mL nutrient broth, the tubes were incubated at 30°C for 24 h and then poured aseptically into 250 mL flasks containing 100 mL of nutrient broth and incubated at 30°C for 48 h. The first inoculation was applied simultaneously with the transplantation of the germinated seeds by adding 120 mL of the bacterial suspension to each pot and the second inoculation was applied 4 weeks after transplantation. One week after transplantation, the pots were regularly irrigated with Hoagland solution [25]. From the fifth week after transplantation, and for a 3-week duration, the irrigation solutions (Hoagland solution) contained NaCl treatments at different concentrations: 0 mM L<sup>-1</sup>, 100 mM L<sup>-1</sup>, and 150 mM L<sup>-1</sup>. We measured the total fresh weight of plants using a precision scale. The chlorophyll content was measured following the method of Francis et al. [26], the proline content following Bergman and Loxley [27], and the K<sup>+</sup> and Na<sup>+</sup> cations were measured using the method described by Lagerkvist et al. [28]. The experiment was conducted according to a completely randomized design with four replicates. STAT BOX v6.40, used to perform ANOVA based on Student–Newman–Keuls test with a significance threshold of  $p = 0.05$ , and Microsoft Excel 2013 was used for the graphics.

## Results

### Isolation, selection, and identification of *Pseudomonas* spp. strains

The macroscopic and microscopic analyzes of the isolates extracted from the rhizosphere revealed seven strains belonging to the genus *Pseudomonas*: P1, P6, and P7 from the saline soil and P11, P13, P14, and P15 from the vineyard soil. We chose P1, P7, and P15 as high-performance strains from the seven isolates as screened by the performance test results shown in Tab. 1. According to the API 20NE gallery identification catalog, isolates P1 and P7 are similar to strains of *P. fluorescens* and P15 was assigned as *P. putida*.

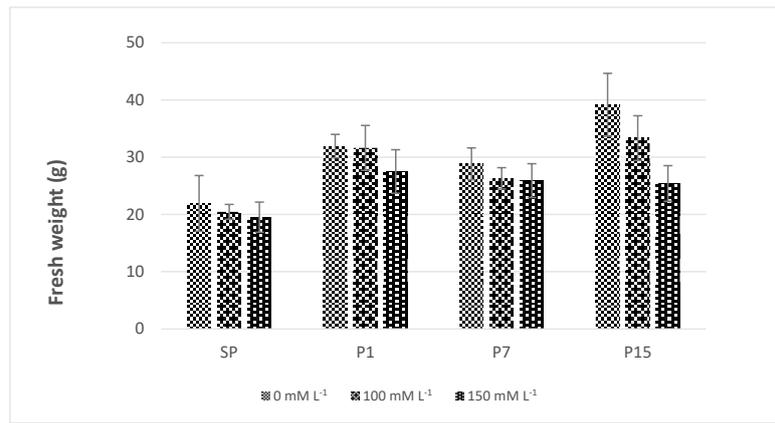
**Tab. 1** Characterization of isolated bacterial strains.

Bacterial isolates	IAA production	Pyoverdine production	Test of resistance to different NaCl doses after 72 h			
			7%	8%	9%	10%
P1	++	++	++	-	-	-
P6	-	-	-	-	-	-
P7	++	++	++	++	++	++
P11	-	+	++	-	-	-
P13	+	+	++	-	-	-
P14	-	+	++	-	-	-
P15	++	++	++	++	++	+

Note: “-” means no production or growth; “+” means weak production or growth; “++” means abundant production or growth.

### Fresh weight

The results showed that salinity has an inversely proportional effect on the fresh weight of plants in the presence and absence of bacterial inoculation. Compared to the 0 mM L<sup>-1</sup> dose, saline doses of 100 and 150 mM L<sup>-1</sup> caused a biomass decrease of 6.73% and 10.95%, respectively, for the noninoculated plants. Saline doses also decreased the biomass of inoculated plants, the P1 strain decreased by 0.95% and 13.86% for the 100 and 150 mM L<sup>-1</sup>, respectively, 8.76% and 10.16% for the P7 inoculum, and 14.85% and 35.10%



**Fig. 1** The effect of salinity and inoculation of *Pseudomonas* strains (P1, P7, P15) versus a control (SP) on fresh weight of *Vicia faba* plants.

for the P15 inoculum (Fig. 1). However, bacterial inoculation with each of the strains significantly increased (Tab. 2) the fresh weight of plants at all levels of saline treatment. The P1 inoculum increased the biomass at a rate of 45.61%, 54.63%, and 40.85% for the 0, 100, and 150 mM L<sup>-1</sup> doses, respectively. The P7 inoculum caused an increase of 31.51%, 28.66%, and 32.68% for the 0, 100, and 150 mM L<sup>-1</sup> treatments, respectively, and the rate of increase was 78.54%, 63.02%, and 30.13% for the P15 inoculum.

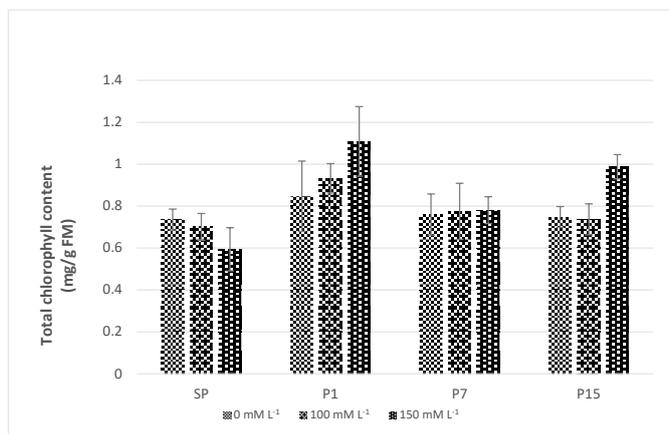
**Total chlorophyll content (a + b)**

The results of noninoculated plants showed an 18.39% increase in total chlorophyll at a saline dose of 100 mM L<sup>-1</sup>, whereas the application of a 150 mM L<sup>-1</sup> saline dose caused a significant (*p* < 0.05) decrease of 21.94% compared to the nonsaline treatment (0 mM L<sup>-1</sup>) (Fig. 2). The results of the plants inoculated with P1 revealed that the effect of salinity on the total chlorophyll content was proportional to the saline concentration, with the 100 and 150 mM L<sup>-1</sup> treatments causing an increase of 10.15% and 25.02%, respectively compared to the 0 mM L<sup>-1</sup> treatment. In parallel, inoculation with the P1

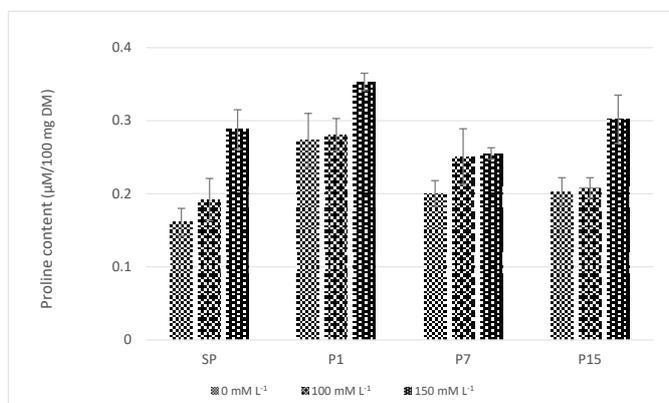
**Tab. 2** The effect of salinity and bacterial inoculation with *Pseudomonas* strains (P1, P7, and P15) on the fresh weight, proline content, chlorophyll content, and K<sup>+</sup>/Na<sup>+</sup> ratio of *Vicia faba*.

Bacterial inoculum	NaCl treatment (mM L <sup>-1</sup> )	Fresh weight (g)	Proline content (µM/100 mg DM)	Chlorophyll content (mg/g FM)	K <sup>+</sup> /Na <sup>+</sup> ratio
SP	0	21.938 ± 4.87 <sup>def</sup>	0.161 ± 0.019 <sup>c</sup>	0.736 ± 0.05 <sup>cd</sup>	2.275 ± 0.667 <sup>b</sup>
SP	100	20.46 ± 1.301 <sup>ef</sup>	0.192 ± 0.029 <sup>c</sup>	0.701 ± 0.064 <sup>cd</sup>	1.029 ± 0.177 <sup>c</sup>
SP	150	19.535 ± 2.631 <sup>f</sup>	0.288 ± 0.027 <sup>b</sup>	0.594 ± 0.103 <sup>d</sup>	0.893 ± 0.14 <sup>c</sup>
P1	0	31.943 ± 2.051 <sup>bc</sup>	0.273 ± 0.037 <sup>b</sup>	0.847 ± 0.168 <sup>bc</sup>	2.101 ± 0.313 <sup>b</sup>
P1	100	31.638 ± 3.916 <sup>bc</sup>	0.281 ± 0.022 <sup>b</sup>	0.933 ± 0.07 <sup>bc</sup>	1.127 ± 0.196 <sup>c</sup>
P1	150	21.515 ± 3.805 <sup>bcde</sup>	0.353 ± 0.012 <sup>a</sup>	1.109 ± 0.166 <sup>a</sup>	1.119 ± 0.081 <sup>c</sup>
P7	0	28.85 ± 2.783 <sup>bcd</sup>	0.200 ± 0.018 <sup>c</sup>	0.76 ± 0.098 <sup>cd</sup>	2.874 ± 0.295 <sup>a</sup>
P7	100	26.323 ± 1.859 <sup>bcdef</sup>	0.251 ± 0.038 <sup>b</sup>	0.775 ± 0.134 <sup>cd</sup>	1.05 ± 0.151 <sup>c</sup>
P7	150	25.92 ± 2.953 <sup>bcdef</sup>	0.255 ± 0.008 <sup>b</sup>	0.781 ± 0.064 <sup>cd</sup>	0.68 ± 0.146 <sup>c</sup>
P15	0	39.168 ± 5.491 <sup>a</sup>	0.203 ± 0.019 <sup>c</sup>	0.748 ± 0.05 <sup>cd</sup>	2.938 ± 0.345 <sup>a</sup>
P15	100	33.353 ± 3.902 <sup>b</sup>	0.208 ± 0.014 <sup>c</sup>	0.737 ± 0.074 <sup>cd</sup>	2.024 ± 0.115 <sup>b</sup>
P15	150	25.42 ± 3.118 <sup>cdef</sup>	0.302 ± 0.033 <sup>b</sup>	0.988 ± 0.058 <sup>ab</sup>	0.868 ± 0.196 <sup>c</sup>

SP – treatment without bacterial inoculation; DM – dry matter; FM – fresh matter. The SP treatment was not inoculated. Results are presented as the mean ± standard deviation; lowercase letters denote homogeneous groups.



**Fig. 2** The effect of salinity and bacterial inoculation with *Pseudomonas* strains (P1, P7, and P15) on the total chlorophyll content of *Vicia faba* plants. SP is a control that was not inoculated.



**Fig. 3** The effect of salinity and bacterial inoculation of *Pseudomonas* strains (P1, P7, and P15) on proline content in *Vicia faba* plant. SP was not inoculated.

strain caused a significant elevation (Tab. 2) in total chlorophyll of 11.30%, 3.55%, and 78.28% for the 0, 100, and 150 mM L<sup>-1</sup> treatments.

For the inoculation with the P7 strain, the results showed that the accumulation of total chlorophyll in the inoculated plants is proportional to the saline concentration applied, with a slight increase of 1.97% and 2.76% induced by 100 and 150 mM L<sup>-1</sup> treatments, respectively. However, the effect of P7 inoculation on total chlorophyll content was negatively affected by the 0 and 100 mM L<sup>-1</sup> treatments with a reduction of 0.13% and 13.980%, respectively, and for the 150 mM L<sup>-1</sup> treatment, the content was increased by 31.48% compared to the control.

Plants inoculated with the P15 strain and stressed with a 100 mM L<sup>-1</sup> saline dose had a 16.84% decrease in total chlorophyll. The 150 mM L<sup>-1</sup> saline treatment caused a 32.09% increase in total chlorophyll compared to the 0 mM L<sup>-1</sup> treatment. In comparison with the results of the noninoculated plants, the P15 inoculation decreased the total chlorophyll content by 1.71% and 30.97% for 0 and 100 mM L<sup>-1</sup> treatments, respectively, while total chlorophyll increased by 66.33% for the 150 mM L<sup>-1</sup> treatment.

#### Proline content in the aerial part

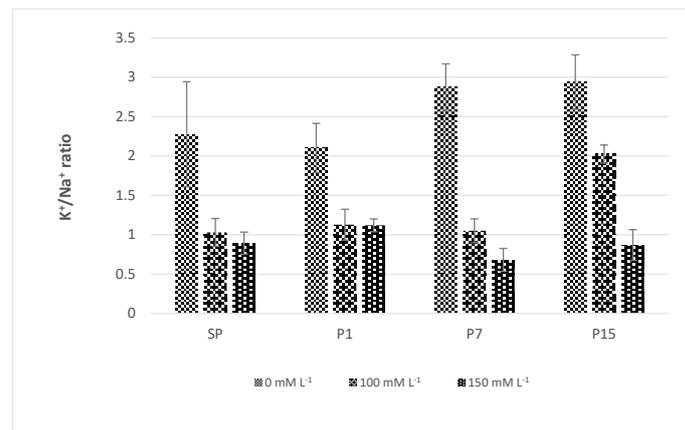
The results of the noninoculated samples showed that the effect of salinity on the accumulation of proline in the aerial part of the bean is proportional to the concentration of the saline treatment, whereas a significant increase (Tab. 2) of 255.90% and 19.25% was observed for the 150 and 100 mM L<sup>-1</sup> saline doses, respectively, compared to the 0 mM L<sup>-1</sup> dose (Fig. 3).

In the group of plants inoculated with the P1 strain, the effect of salt stress on the proline content

in the aerial part of the bean was proportional to the concentration of the saline treatment, with proline content increases of 2.93% and 29.30% for the 100 and 150 mM L<sup>-1</sup> treatments, respectively, compared to the 0 mM L<sup>-1</sup> treatment. Comparison of the results of this group with those of the noninoculated group revealed a significant increase of 69.56% and 46.35% in the proline content for the 0 and 100 mM L<sup>-1</sup> treatments, respectively, whereas a decrease of 38.39% was observed for the 150 mM L<sup>-1</sup> treatment.

The plants inoculated with the P7 strain displayed a proportional effect of salinity on the accumulation of proline in the aerial part of the bean. There was a significant increase of 25.5% and 27.5% (Tab. 2) in the proline content of the plants stressed with 100 and 150 mM L<sup>-1</sup> treatments, respectively, compared to the 0 mM L<sup>-1</sup> dose. In comparison with noninoculated plants, the plants inoculated with the P7 strain showed an increase of 24.22% and 30.72% in the proline content for the 0 and 100 mM L<sup>-1</sup> saline doses, respectively, and a decrease of 55.49% for the 150 mM L<sup>-1</sup> saline dose.

The values of the proline content in the plants inoculated with the P15 bacterial strain indicated that the accumulation of proline was proportional to the saline concentration. The 100 and 150 mM L<sup>-1</sup> treatments caused significant accumulation of proline content of 2.46% and 48.76%, respectively, compared to the 0 mM L<sup>-1</sup> treatment. Comparing the results of the plants inoculated with the P15 strain with those not inoculated (SP), the effect of the P15 bacterial inoculum on the proline accumulation was a significant increase of 26.08% and 8.33% for the 0 and 100 mM L<sup>-1</sup> treatments, respectively, and a decrease of 47.29% for the 150 mM L<sup>-1</sup> treatment.



**Fig. 4** The effect of salinity and bacterial inoculation with *Pseudomonas* strains (P1, P7, and P15) on the K<sup>+</sup>/Na<sup>+</sup> ratio of *Vicia faba* plants. The SP treatment was not inoculated.

### K<sup>+</sup>/Na<sup>+</sup> ratio

Compared with unstressed *Vicia faba* plants (treated with 0 mM L<sup>-1</sup> solution), the 100 and 150 mM L<sup>-1</sup> saline doses significantly decreased the K<sup>+</sup>/Na<sup>+</sup> ratio in the noninoculated plants by 62.91% and 57%, respectively (Fig. 4). The results showed that the K<sup>+</sup>/Na<sup>+</sup> ratio is inversely proportional to the saline concentrations, with the 100 and 150 mM L<sup>-1</sup> treatments causing a significant reduction (Tab. 2) of 46.35% and 46.73%, respectively, comparatively to the 0 mM L<sup>-1</sup> treatment. In parallel, inoculation with the P1 strain was accompanied by a significant decrease of 24.28% and 6.20% in the K<sup>+</sup>/Na<sup>+</sup> ratio for the 0 and 150 mM L<sup>-1</sup> saline doses, respectively; however, there was an increase of 9.52% for the 100 mM L<sup>-1</sup> dose compared to noninoculated plants.

The results obtained showed that the K<sup>+</sup>/Na<sup>+</sup> ratio in plants inoculated with P7 is inversely proportional to the saline doses applied, with a considerable reduction of 63.46% and 76.33% for the 100 and 150 mM L<sup>-1</sup> doses, respectively. Compared to noninoculated plant results, the effect of the P7 inoculation on the K<sup>+</sup>/Na<sup>+</sup> ratio was positively demonstrated for the 0 and 100 mM L<sup>-1</sup> treatments with a significant increase of 35.67% and 20.40% respectively, while the K<sup>+</sup>/Na<sup>+</sup> ratio was reduced by 43% for the 150 mM L<sup>-1</sup> treatment.

In comparison with the results for the noninoculated plants, the P15 inoculation increased the K<sup>+</sup>/Na<sup>+</sup> ratio by 5.87% and 96.69% for the 0 and 100 mM L<sup>-1</sup> treatments, respectively. However, the K<sup>+</sup>/Na<sup>+</sup> ratio decreased by 27.24% for the 150 mM L<sup>-1</sup> treatment. The effect of saline stress on the K<sup>+</sup>/Na<sup>+</sup> ratio is inversely proportional to the concentration of the saline treatment, with a reduction of 31.10% and 70.45% in the K<sup>+</sup>/Na<sup>+</sup> ratio observed for the 100 and 150 mM L<sup>-1</sup> treatments, respectively, compared to the 0 mM L<sup>-1</sup> dose.

## Discussion

### Fresh weight

The results obtained in the bean plants inoculated with the P1, P7, and P15 strains and those not inoculated revealed a reduction in fresh weight induced by the 100 and 150 mM L<sup>-1</sup> saline doses compared to the 0 mM L<sup>-1</sup> nonsaline treatment. Huge reductions in growth are caused by salt concentrations in the irrigation solution [29]. Salt stress reduces the dry matter of roots, stems, leaves, and the leaf surface due to the direct and indirect effects of salt ion toxicity that cause soil–plant osmotic imbalance [30]. In response to salt stress detection, plants increase ethylene production [29,31–33]. Ethylene affects different vegetative growth phases in plants, leading to an overall reduction in growth [34], and it can inhibit the elongation of stems and plant roots [35,36].

Our results also show that inoculation with the P1, P7, and P15 strains significantly increased plant growth under both saline and nonsaline conditions. Inoculation of stressed plants with bacteria containing ACC (1-aminocyclopropane-1-carboxylate)-deaminase may reduce ethylene concentration [29]. Many PGPRs produce the enzyme ACC-deaminase and metabolize ACC, the precursor for ethylene synthesis of plants, thereby reducing the inhibition of root growth by stress-induced ethylene [37–39]. *Pseudomonas extremorientalis* TSAU20 is able to reduce salt stress in wheat grown in saline soil [18]; the improvement effect of PGPRs on plant growth under saline conditions has been demonstrated in different species of plants such as tomato, pepper, canola, beans, and lettuce [12,40–43]. *Pseudomonas putida* Rs-198 secretes IAA, which enhances plant growth, and alleviates the effect of growth inhibitors by decreasing the abscisic acid (ABA) content of plants [44–46].

However, we observed a proportional reduction in the fresh weight of inoculated plants with increased saline concentration, indicating that salinity has inhibitory effects on the development of nodulation and colonization of inoculation strains. Several studies have proven the inhibitory effect of salt stress on the association of plants with symbiotic bacteria. Salt stress inhibits the growth, nodulation, and nitrogen fixation of several legumes, such as soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) [47,48]. An explanation for reduced legume growth may be that salt stress causes failure of the infection and nodulation processes [49].

### Chlorophyll content

According to the results, an optimum content of total chlorophyll was found in the noninoculated *Vicia faba* plants treated with the 100 mM L<sup>-1</sup> saline dose, whereas the 150 mM L<sup>-1</sup> saline treatment reduced the total chlorophyll content. Salinity decreases photosynthesis and degrades chlorophyll and chlorophyll–protein complexes [50,51]; chlorophyll concentrations were significantly reduced by salinity treatments due to the suppression of the specific enzymes responsible for the synthesis of photosynthetic pigments [52,53], or the antagonistic effect of Na<sup>+</sup> on the absorption of minerals (for example, Mg) entering into the synthesis of photosynthetic pigments, thereby reducing the chlorophyll concentration [51,54].

The chlorophyll content in the plants inoculated with the P1, P7, and P15 strains under salt stress are higher than that in the nonsaline treatment. The inoculated plants under salt stress reached higher levels of photosynthetic capacity than that of the unstressed plants. Compared with the noninoculated plants, P1 inoculation showed an increase in chlorophyll under saline and nonsaline conditions; chlorophyll (*a*) increased in the 150 mM L<sup>-1</sup> stressed plants inoculated with the P7 and P15 strains. Our results agree with several previous studies reporting that bacterial inoculation increases chlorophyll in leaves [55–57].

### Proline content

For the present study, the results obtained show that proline accumulates in *Vicia faba* plants in proportion to the NaCl treatment concentrations, as recorded in the presence and absence of the P1, P7, and P15 bacterial inoculation, there was a higher proline content in plants exposed to salt stress than in plants not stressed by salinity. These results are consistent with those of several authors, who observed an increase in proline [58,59]. The accumulation of proline in plants is an indicator of general stress tolerance or salinity tolerance, as it maintains osmotic adjustment and protects intracellular macromolecules against dehydration and also serves as a hydroxyl radical scavenger [60,61]. Proline accumulation is one of the most frequently reported modifications induced by hydric and salt stress to plants and is often considered to be involved in stress resistance mechanisms. Proline accumulation is a sensitive physiological index of plant response to salt stress and other stress [62]. It is also one of the adaptive strategies triggered by the plant against environment constraints [63]. Under salt stress, plants accumulate some organic components (such as proline and soluble sugar) and inorganic ions in order to maintain higher osmotic adjustment [64].

It has also been found that in inoculated plants, proline is higher than in noninoculated plants, under saline a nonsaline treatment, which explains the effect of bacterial inoculation on proline accumulation and consequently on plant tolerance to different stresses. The leaf proline levels increased in response to inoculation with microorganisms [65]. Proline accumulation was significantly lower for noninoculated plants and significant proline accumulation was found in the leaves of plants inoculated with *Piriformospora indica*. *Azospirillum* can also accumulate proline and glutamate in response to NaCl and limit the influx of Na<sup>+</sup> in roots [66]. An increase in proline and total soluble sugars was observed in plants treated with PGPRs, which have probably led to a significant contribution to the promotion of plant growth under salt stress, by increasing several metabolic defense strategies [65], and several authors have confirmed the effect of bacterial inoculation on proline increase under saline conditions [67–69].

#### Na<sup>+</sup>/K<sup>+</sup> ratio

Salinity causes an increase in Na<sup>+</sup> concentration and a decrease in K<sup>+</sup>, which reduces the K<sup>+</sup>/Na<sup>+</sup> ratio in proportion with the increase in salt stress; the reduction in K<sup>+</sup> concentration in plants under salt stress may increase the deleterious effects of salinity on growth and yield [70]. High NaCl concentrations in the soil solution may decrease the K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios in plants, which would then be more susceptible to osmotic and specific ion alterations as well as nutritional disturbances, which consequently lead to yield and quality reduction [71,72]. Salinity increased foliar Na<sup>+</sup> and Ca<sup>2+</sup> concentrations and decreased K<sup>+</sup> in the leaves of lettuce [4]. Na<sup>+</sup> exclusion and K<sup>+</sup> influx are the most important plant strategies for relieving salt stress [73–75]. These results are consistent with those obtained in the present experiment, where we observed that saline treatments induce a significant increase in Na<sup>+</sup> concentration and a decrease in K<sup>+</sup> in inoculated and noninoculated plants; a significant reduction in the K<sup>+</sup>/Na<sup>+</sup> ratio under saline treatments in the presence and absence of inoculation was also noted.

Compared with noninoculated plants, inoculation with the P1, P7, and P15 strains caused a decrease in Na<sup>+</sup> concentration in plants treated with 0 and 100 mM L<sup>-1</sup> NaCl solutions, and therefore a reduction in the K<sup>+</sup>/Na<sup>+</sup> ratio. This indicates an improved effect of inoculation with the selected bacterial strains on the reduction in the salt stress effect, this osmotic regulation is achieved by reducing the level of Na<sup>+</sup> toxic ion availability in plants. Inoculation with exopolysaccharide-producing bacteria can reduce Na<sup>+</sup> influx in plant roots [76]. Inoculation with *Bacillus subtilis* GB03 could also improve the level of salt tolerance in *Arabidopsis thaliana* by regulating the HKT1 potassium transporter [77]. Inoculation with PGPR strains helps to relieve salt stress by the induction of certain genes and polypeptides, or regulation of the HKT1 potassium transporter [78]; PGPRs affect HKT1, which has an effect on the adjustment of the Na<sup>+</sup> and K<sup>+</sup> levels, it has also been suggested that PGPR increases the uptake of mineral ions by plants, via proton pump ATPase stimulation [79]. Ashraf et al. [76] found that Na<sup>+</sup> accumulation in wheat decreases in the presence of PGPRs, improved exopolysaccharide (EPS) production by PGPRs can help plants tolerate salt stress by reducing the availability of Na<sup>+</sup> ions at the root level. A decrease in Na<sup>+</sup> availability can alleviate salt stress for wheat and cotton plants [76,80,81].

#### Conclusion

This study aimed to investigate the role of rhizobacterial *Pseudomonas* inoculation in the clearance of salt stress effects on the growth and physiology of *V. faba*. The results showed that the 100 and 150 mM L<sup>-1</sup> saline treatments induced a regression in the fresh weight of *V. faba* plants inoculated with P1, P7, and P15 and the noninoculated plants. In the absence of bacterial inoculation, the optimum content of total chlorophyll releasable is linked to treatment with 100 mM L<sup>-1</sup> of NaCl, whereas treatment with 150 mM L<sup>-1</sup> reduced the total chlorophyll content. Proline also accumulates in *V. faba* plants in proportion to the saline concentration in the presence and absence of bacterial inoculation. Saline treatments induced a significant increase in Na<sup>+</sup> and

a decrease in K<sup>+</sup> in inoculated and noninoculated plants. We also noted a significant reduction in the K<sup>+</sup>/Na<sup>+</sup> ratio under saline treatments in the presence and absence of bacterial inoculation. However, inoculation with strains P1, P7, and P15 caused a decrease in Na<sup>+</sup> in plants treated with 0 and 100 mM L<sup>-1</sup> NaCl, this increased the K<sup>+</sup>/Na<sup>+</sup> ratio, indicating that inoculation by selected bacterial strains improves the tolerance of plants to salinity. This study provides insights into the effect of salinity on plant growth and its complications.

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#### Oddziaływanie niektórych bakterii ryzosferowych *Pseudomonas* na wzrost i parametry fizjologiczne roślin bobu (*Vicia faba*) w warunkach zasolenia

##### Streszczenie

Oddziaływanie stresu solnego na wzrost i rozwój roślin jest zróżnicowane. Wynika bowiem zarówno z wpływu zasolenia na potencjał wodny, fotosyntezę i pobieranie składników pokarmowych jak i zmian w procesach fizjologicznych i biochemicznych. Oddziaływanie bakterii promujących wzrost roślin (PGPR), szczególnie jako czynników wzmacniających wzrost i rozwój roślin w warunkach zasolenia, należy do coraz częściej podejmowanych tematów badawczych. Głównym celem badań było określenie reakcji roślin bobu w warunkach zasolenia i ocena roli wyselekcjonowanych izolatów *Pseudomonas* spp. w poprawie tolerancji roślin na stres solny. W badaniach wykorzystano trzy izolaty *Pseudomonas* spp. (otrzymane odpowiednio: P1 i P7 z gleby zasolonej i P15 z gleby spod uprawy winorośli). Rośliny *Vicia faba* (OTONO) inokulowano wymienionymi izolatami, a następnie podlewano roztworem NaCl w dwóch stężeniach, tj. 100 i 150 mM L<sup>-1</sup> oraz wodą bez NaCl. Wykazano, że zasolenie obniżyło wielkość świeżej masy roślin, całkowitą zawartość chlorofilu i stosunek jonów Na<sup>+</sup>/K<sup>+</sup>, ale zwiększyło akumulację proliny zarówno w inokulowanych jak i nieinokulowanych roślinach. Traktowanie roślin *V. faba* izolatami P1, P7 i P15 znacznie zwiększyło produkcję świeżej biomasy w obecności i przy braku stresu solnego oraz pozytywnie wpłynęło na akumulację proliny i stosunek Na<sup>+</sup>/K<sup>+</sup>. Rośliny inokulowane zawiesiną bakterii *Pseudomonas* spp. charakteryzowały się większą całkowitą zawartością chlorofilu we wszystkich kombinacjach doświadczalnych z użyciem roztworu soli w porównaniu do kombinacji kontrolnej. Spośród badanych *Pseudomonas* spp. izolat P1 był najbardziej efektywny.