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Characterization of Plant Growth-Promoting Traits and Inoculation Effects on *Triticum durum* of Actinomycetes Isolates under Salt Stress Conditions

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Abstract: This study aimed to characterize the halotolerant capability, in vitro, of selected actinomycetes strains and to evaluate their competence in promoting halo stress tolerance in durum wheat in a greenhouse experiment. Fourteen isolates were tested for phosphate solubilization, indole acetic acid, hydrocyanic acid, and ammonia production under different salt concentrations (i.e., 0, 0.25, 0.5, 0.75, 1, 1.25, and 1.5 M NaCl). The presence of 1-aminocyclopropane-1-carboxylate deaminase activity was also investigated. Salinity tolerance was evaluated in durum wheat through plant growth and development parameters: shoot and root length, dry and ash-free dry weight, and the total chlorophyll content, as well as proline accumulation. In vitro assays have shown that the strains can solubilize inorganic phosphate and produce indole acetic acid, hydrocyanic acid, and ammonia under different salt concentrations. Most of the strains (86%) had 1-aminocyclopropane-1-carboxylate deaminase activity, with significant amounts of α-ketobutyric acid. In the greenhouse experiment, inoculation with actinomycetes strains improved the morpho-biochemical parameters of durum wheat plants, which also recorded significantly higher content of chlorophylls and proline than those uninoculated, both under normal and stressed conditions. Our results suggest that inoculation of halotolerant actinomycetes can mitigate the negative effects of salt stress and allow normal growth and development of durum wheat plants.

Keywords: PGPB; actinomycetes; 1-aminocyclopropane-1-carboxylate deaminase; durum wheat; salt stress; halotolerance

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1. Introduction

Agriculture is affected by climate change, excessive uses of chemicals, exploitation of groundwater in areas close to the sea, and insufficient drainage, which increased soil exposure to salt stress [1]. Salinity is a major problem affecting agricultural productivity, especially in arid and semi-arid soils [1–5]. Salinity degrades soil fertility and interferes with the normal development of plants, severely affecting crop productivity [3,6,7]. High salt concentration affects plant growth also by disturbing physiological and biochemical functioning [8]. The decrease in the acquisition of nutrients and the accumulation of Na^+ ions [9] causes osmotic stress and closure of the stomata, which reduces the leaf water level and alters CO_2 uptake and photosynthesis [4,10]. Salinity affects plant organization and the various plant organs; causes turgor loss and membrane dysfunction, which consequently lead to ionic toxicity and cellular dehydration [3,5]. Furthermore, the decrease in water

status due to salinity alters photosynthesis causing the generation of reactive oxygen species (ROS) [11,12].

Plant-microorganisms associations help plants overcome various environmental stresses [13] and are essential for maintaining soil health [3]. Plant growth-promoting bacteria (PGPB) are recognized as active against the negative effects of environmental stress, thanks to the induced systemic tolerance (IST) [3,14–16]. The IST mechanisms induced by PGPB includes [1,17,18]: solubilization of nutrients; activation of stress-responsive genes; regulation of phytohormone synthesis (e.g., 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity); promotion of antioxidant defenses; regulation of osmotic balance (e.g., proline accumulation), turgor pressure, ion homeostasis, and production of volatile organic compounds. Moreover, halophilic/halotolerant PGPB can survive in the presence of high salt concentrations [19]. Halophily/halotolerance and IST mechanisms induced by PGPB allow plants to survive under high salt concentrations [2,19–23].

Several studies have demonstrated the role of PGPB in salt stress mitigation, mainly in *Bacillus* spp. and *Pseudomonas* spp. strains [24–26]. However, studies on the halophilic/halotolerant microbiota are still needed to provide new perspectives in the design of bioformulations useful in agriculture [26]. Several studies have demonstrated that Actinobacteria are one of the most common class found in saline soils [27,28].

Our previous studies have shown that several actinomycetes isolated from Algerian saline soils (salt tolerance up to 10% (i.e., 1.7 M)) [29] have good in vitro and in planta (*Solanum lycopersicum* L.) plant growth-promoting (PGP) traits under normal conditions [30]. In this study, we advanced the hypothesis that these strains could be valid agents to salt stress tolerance. To test this hypothesis, we evaluated halotolerance traits in vitro with different PGP activities (i.e., phosphate solubilization, indole acetic acid production, hydrocyanic acid, and ammonia production under saline stress and ACC deaminase activity). Then, a greenhouse experiment was carried out in durum wheat (*Triticum durum* Desf.) to evaluate the inoculation effects on plant growth and in the mitigation of salt stress.

2. Materials and Methods

2.1. Actinomycetes Strains

The actinomycetes strains were isolated from two saline sites in the northeast region of Algeria, Ezzemoul sebkha—35°53′14″ North; 06°30′20″ East; pH 8.6; EC (electrical conductivity) 1600 µS cm^{-1}; organic matter 6.0%; moisture and organic matter 6.0%; sand 52.8%; slit 22.4%; clay 24.8%—and Djendli sebkha—35°43′15″ North; 06°32′23″ East; pH 8.0; EC 1101 µS cm^{-1}; organic matter 6.2%; moisture and organic matter 5.8%, sand 31.0%; slit 32.7%; clay 36.3% [29,30]. The soil samples were taken according to the Pochon and Tardieux method [31]. Briefly, the samples were taken by removing the first five centimeters of the top soil layer. Then, around 100 g of soil from the underlying layer were taken and transported into sterile tubes to the laboratory [31].

The spore production of each strain was obtained by cultivation on the International Streptomyces Project-2 (ISP-2) agar medium at 30 °C for 7 days. The spore suspensions were prepared by adding sterile distilled water into the plates; the mixtures were filtrated through a syringe containing hydrophilic cotton to eliminate the mycelia. Spore concentrations were adjusted spectrophotometrically to a final density of 10^6 spores mL^{-1}. The spore suspensions were utilized differently, depending on the test carried out (descriptions given in each paragraph).

2.2. Estimation of PGP Traits under Salt Stress

The actinomycetes strains were studied for different PGP traits with the addition of various salt concentrations (i.e., 0, 0.25, 0.5, 0.75, 1, 1.25, and 1.5 M NaCl); phosphate solubilization, indole acetic acid (IAA), hydrocyanic acid (HCN), and ammonia (NH_3) production. Based on PGP test carried out, different procedures and media were utilized.

2.2.1. Hydrocyanic Acid and Ammonia Production

The cyanogenic activity was detected on Trypticase Soy Agar medium (TSA) added with glycine (4.4 g L^{-1}). A Whatman paper was soaked in a solution of picric acid (0.5%) and sodium carbonate (2%) and was placed on the lid of each inoculated plate. The Petri dishes were sealed with parafilm and incubated at 30 °C for 7 days. After incubation, a change in the Whatman paper's color to orange or brown is considered as a positive result [32]. The ammonia production (NH_3) was evaluated in peptone water, change of color from yellow to brown after addition of Nessler's reagent has been marked as a positive result [33].

2.2.2. Phosphate Solubilization Ability

Phosphate solubilization was evaluated on 5 mL of Pikovskaya (PVK) liquid medium [34], inoculated with 100 µL of each spore suspension of the different actinomycetes strains. Broth cultures were kept growing at 30° for 7 days with moderate agitation (150 rpm). The quantification of soluble phosphorus was determined according to the Olsen and Sommers method [35] and the results were expressed as µg PO_4^{3-} mL^{-1}.

2.2.3. Production of Indole-3-Acetic Acid

For IAA production, the spore suspension of each strain (100 µL) was inoculated in 5 mL of the National Botanical Research Institutes' phosphate growth medium (NBRIP) supplemented with tryptophan (0.2%) [36–40]. The broth cultures were kept to grow at 30 °C for 7 days at 150 rpm. After incubation, the cultures were centrifuged at 3000 rpm for 20 min, then 1 mL of the clear supernatant was mixed with 4 mL of the Salkowski reagent [41]. The mixture was incubated at 37 °C for 30 min in the dark and optical density was read at 530 nm (SPEKOL 1300 UV VIS spectrophotometer, Analytik Jena, Jena, Germany). IAA (Sigma, St. Louis, MO, USA) was used as standard ($y = 0.0089x + 0.0113$; $R^2 = 0.9975$) and results were expressed as µg IAA mL^{-1}.

2.3. Estimation of 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase Activity

The estimation of ACC deaminase activity was carried out following the procedure described by Brígido et al. [42]; 200 µL of each spore suspension was inoculated in 15 mL of ISP-2 liquid medium and incubated for 3 days at 30 °C with shaking (200 rpm). The cultures were then centrifuged and washed twice with 10 mL of DF (Dworkin and Foster) salts minimum medium without a nitrogen source [43]. Cell pellets were resuspended in 15 mL of minimum DF salt medium with 3 mM of ACC and incubated for 3 days at 30 °C with shaking, followed by centrifugation and washing step with 5 mL of 0.1 M Tris-HCl (pH 7.6). The cell suspensions were transferred into 1.5 mL microcentrifuge tubes, the supernatant was removed after centrifugation at 10,000× *g* for 1 min and the cell pellet was used for enzymatic activity test. The pellet of the different actinomycetes strains was resuspended in 400 µL of 0.1 M Tris—HCl (pH 8.0) with 20 µL of toluene. Moreover, 50 µL of cell lysate from each strain was distributed in each of three microtubes, where 5 µL of ACC (0.5 M) was added in two tubes, while the third tube served as a negative control. Another negative control was also prepared to contain 50 µL of 0.1 M Tris-HCl (pH 8.0) and 5 µL of 0.5 M ACC. After adding the ACC, the cell suspensions were vortexed for 5 s, after that, all the tubes were incubated at 30 °C for 30 min. After the incubation, 500 µL of 0.56 M HCl was added to each tube then vortexed for 5 s. The cells were centrifuged for 5 min at 10,000× g at room temperature. The solution of α-ketobutyrate (Sigma) in 0.1 M TRIS-HCl (pH 8.0) was used as standard. Absorbances of the reaction mixtures were detected at 540 nm (Cary Bio 50 UV VIS spectrophotometer, Varian, Palo Alto, CA, USA). The ACC deaminase activity of the strains was determined with a calibration curve of α-ketobutyrate (5, 10, 15, 20, and 25 µmol mL^{-1}). ACC deaminase activity of the strains was expressed as µmol α-ketobutyrate h^{-1} mg protein $^{-1}$. Protein content was estimated according to the Bradford method [44] using Bovine Serum Albumin (BSA) as standard. A

calibration curve with BSA (1.25, 2.5, 5, and 10 µg/mL) was used to determine the total protein concentration of the extracts.

2.4. Greenhouse Experiment on Triticum durum

The experiment was carried on durum wheat (*Triticum durum*) of the Cirta R1 variety, with susceptibilities to abiotic stresses comparable to other *Triticum durum* parental genotypes [45–47]. To obtain optimal germination rate, the seeds were kept in sterile distilled water for 24 h. The seeds were surface sterilized with a 20% (*v*/*v*) sodium hypochlorite solution washed several times with sterile distilled water [5] and left to dry in sterile conditions for 3 h. The inoculation was carried out by immersing the seeds in spore suspensions (10^6 spores mL^{-1}) of the different isolates for 1 h with moderate agitation; seeds dipped in sterile distilled water were used as control (uninoculated) [30]. The experiment was performed as follows: CNT (without PGPB and with/without salt stress), PGPB (with PGPB and with/without salt stress). The inoculated and not- inoculated seeds were sown in pots with three seeds per pot (ø = 10 cm), on which the inner surface was disinfected with ethanol (70%), filled with autoclaved soil (pH 8.27, electrical conductivity 332 µS cm^{-1}, 5% organic matter, humidity rate 0.7%) grown in natural light conditions and irrigated with tap water for 10 days. Each experimental unit consisted of five replications. The plants were then irrigated twice a week with NaCl solution at increasing concentrations 0, 0.25, 0.5, 0.75, 1, 1.25, and 1.5 M during 4 weeks. Not salt stressed plants (0 M NaCl L^{-1}) were irrigated with tap water only [4]. Forty days after sowing (DAS), roots and shoots were collected separately. Shoots and roots lengths (by ruler), fresh weight (FW), dry weight (oven drying at 105 °C for 48 h), and ash-free dry weight (AFDW) were determined [30].

2.4.1. Chlorophyll Measurement

Chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (Chl_{tot}) contents were detected after 40 DAS as described by Arnon [48]. Briefly, 0.5 g of plant leaves or stem tissues from each sample were finely cut and homogenized in 10 mL of 80% acetone and stored at −10 °C overnight in the dark, after centrifugation at 14,000 rpm for 5 min. The absorbance of the supernatant was measured at 663 and 645 nm (SPEKOL 1300 UV VIS spectrophotometer, Analytik Jena) to determine chlorophyll a (Chl_a) and b (Chl_b) and total (Chl_{tot}) following the Equations (1)–(3).

$$Chl_a\ (mg\ L^{-1}) = 12.41\ (OD\ 663) - 2.59\ (OD\ 645) \quad (1)$$

$$Chl_b\ (mg\ L^{-1}) = 22.9\ (OD\ 645) - 4.68\ (OD\ 663) \quad (2)$$

$$Chl_{tot}\ (mg\ L^{-1}) = Chl_a + Chl_b \quad (3)$$

Results were expressed as mg 100 g FW^{-1}. The Chl a: Chl b ratio was calculated to observe the stress effects on this parameter.

2.4.2. Proline Determination

Proline content was detected in fresh leaves after 40 DAS according to the method described by Naidu et al. [49]. Briefly, 0.5 g of the leaves were transferred to tubes containing 5 mL of a methanol:chloroform:distilled water (60:25:15) mixture. The tubes were heated at 60 °C for 2 min and the mixtures were centrifuged at 10,000 rpm for 10 min. To the supernatant (1 mL) was added 4 mL of ninhydrin solution, 4 mL of glacial acetic acid, and 1 mL of distilled water. Then, the mixture was heated at 90 °C for 45 min, cooled to room temperature and absorbance was determined at 520 nm (SPEKOL 1300 UV VIS spectrophotometer, Analytik Jena). Proline (Sigma) was used as reference standard ($y = 0.0314x + 0.0409$; $R^2 = 0.9993$) and the results were expressed as µg proline g fresh $weight^{-1}$ (µg Pro g FW^{-1}).

2.5. Statistical Analysis

All data obtained are the means of three replicates. Data were analyzed by one-way analysis of variance (ANOVA). Separation of the means was carried out with a Tukey's post-hoc test with a probability level of 5%. Data of in vitro and in planta experiments were processed by the Principal Component Analysis (PCA) algorithm. All statistical calculations were carried out using Statistica 10.0 software (Dell Software, Round Rock, TX, USA) and XLSTAT 2014 software (Addinsoft, Paris, France).

3. Results

3.1. Actinomycetes Strains

Fourteen strains of the genus *Streptomyces* and *Nocardiopsis* were investigated for this study.

- *Nocardiopsis aegyptica* (MG597543)—H14;
- *Nocardiopsis aegyptica* (MG597572)—S2;
- *Nocardiopsis alba* (MG597576)—J21;
- *Nocardiopsis dassonvillei subsp. dassonvillei* (MG597514)—D14;
- *Nocardiopsis dassonvillei subsp. dassonvillei* (MG597502)—T45.
- *Streptomyces albidoflavus* (MG597552)—H12;
- *Streptomyces ambofaciens* (MG597599)—J27;
- *Streptomyces anulatus* (MG597579)—J13;
- *Streptomyces iakyrus* (MG597593)—G10;
- *Streptomyces thinghirensis* (MG597560)—K23;
- *Streptomyces thinghirensis* (MG597590)—J4;
- *Streptomyces xantholiticus* (MG597545)—K12;
- *Streptomyces xantholiticus* (MG597582)—G22;
- *Streptomyces xantholiticus* (MG597585)—G33;

3.2. Hydrocyanic Acid and Ammonia Production under Salt Stress

The ability of actinomycetes strains to produce HCN without and under different salt concentrations is shown in Figure 1. In the absence of NaCl, only two strains of *S. xantholiticus* (K12 and G22) and strain of *N. dassonvillei* subsp. *dassonvillei* D14 did not produce HCN. HCN production is negatively affected by the addition of NaCl in the medium, even at low concentrations. Except for two strains of *N. aegyptica* (S2 and H14), at 0.25 M NaCl all strains lost their ability to produce this volatile compound. However, strains S2 and H14 retain their ability to produce HCN up to 1 M NaCl and 1.25 M NaCl, respectively.

In Figure 1, the ability of actinomycetes strains to produce NH_3 at different salt concentrations is shown. All strains shared the ability to produce NH_3 in the absence of NaCl and up to 0.5 M NaCl. At 0.75 M NaCl, only strain *S. anulatus* J13 was negatively affected. At higher salt concentrations, different behaviors were observed, depending on the strain. At 1 M NaCl, 50% of the strains retain their ability to produce NH_3: strains *N. dassonvillei* subsp. *dassonvillei* D14, *S. xantholiticus* G22, *S. albidoflavus* H12, *N. aegyptica* H14, *S. thinghirensis* J4, *N. alba* J21, and *S. thinghirensis* K23. At 1.25 M NaCl, strains *N. dassonvillei* subsp. *dassonvillei* D14, *S. albidoflavus* H12, and *N. aegyptica* H14 were the only NH_3 producers. At 1.5 M NaCl, only *N. aegyptica* strain H14 was able to produce it.

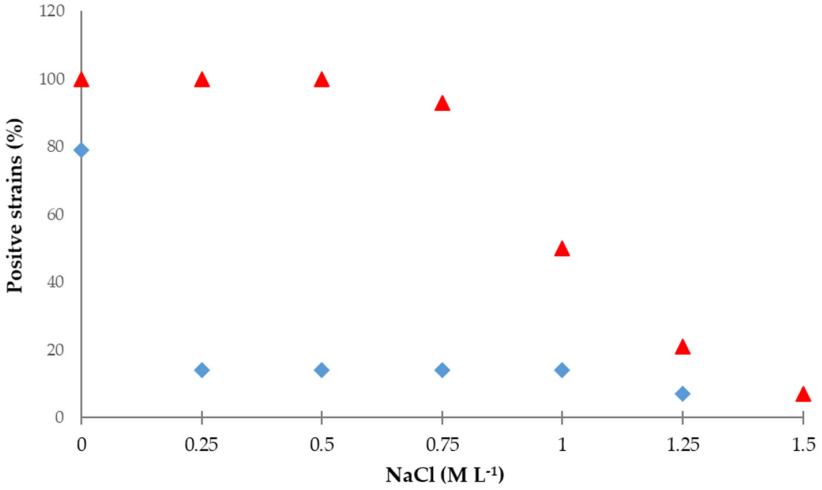

Figure 1. Hydrocyanic acid (HCN, in blue) and ammonia (NH_3, in red) production by the strains under different NaCl concentrations.

3.3. Phosphate Solubilization under Salt Stress

In Table 1, the concentrations of phosphorus solubilized by the strains grown in the different conditions are shown. All strains showed a different capability to solubilize phosphate on PVK liquid medium without NaCl. Best solubilization rate was observed for strain *N. dassonvillei* subsp. *dassonvillei* D14, followed by strains *S. xantholiticus* G22 and *N. aegyptica* H14 ($p < 0.05$). The lowest solubilization ($p < 0.05$) was observed for strain *S. anulatus* J13. For almost all strains, the presence of NaCl in the medium negatively affected the amount of phosphorus solubilization. Conversely, for strains *S. xantholiticus* G33, *S. thinghirensis* J4, *S. anulatus* J13, and *S. xantholiticus* K12, the best values were observed in the presence of NaCl. *S. xantholiticus* G33 showed highest solubilization rate ($p < 0.05$) at 1.5 M NaCl; *S. thinghirensis* J4 at 1 M NaCl; and *S. anulatus* J13 and *S. xantholiticus* K12 at 0.25 M NaCl. Besides *S. xantholiticus* G33, *S. anulatus* J13 and *N. aegyptica* S2 strains also showed considerable concentrations of solubilized phosphorus at 1.5 M NaCl L^{-1}. While in strains *N. dassonvillei* subsp. *dassonvillei* D14, *S. iakyrus* G10, *S. xantholiticus* G22, *S. albidoflavus* H12, *N. aegyptica* H14, *N. alba* J21, *S. ambofaciens* J27 and *N. dassonvillei* subsp. *dassonvillei* T45, a solubilization capability decreased by $\geq 50\%$ ($p < 0.05$) at 1.5 M NaCl. However, for these strains, a good solubilization activity was kept.

3.4. Indole Acetic Acid Production under Salt Stress

In Table 2, the concentrations of IAA produced by the different strains not exposed and exposed to salt are shown. In the absence of salt stress, nine strains could produce IAA. Different behavior was detected among the strains, i.e., some strains (*S. thinghirensis* K23 and *N. aegyptica* S2) did not produce IAA in the conditions tested; strain *S. xantholiticus* G33 produced IAA only without salt and other strains (*S. albidoflavus* H12, *S. thinghirensis* J4, and *N. alba* J21) synthesized IAA only in the presence of NaCl. *N. dassonvillei* subsp. *dassonvillei* D14 and *N. aegyptica* H14 produced IAA even with up to 0.25 and 0.75 M NaCl, respectively. However, in the latter strains, the presence of salt decreased IAA production. *N. dassonvillei* subsp. *dassonvillei* T45—that tolerates salinity up to 1.25 M NaCl and up to 0.5 M NaCl—was able to produce IAA amounts similar ($p > 0.05$) to the control without salt stress (0 M NaCl). In the other strains, IAA production was higher in the presence of NaCl than without. In particular, the highest IAA production was recorded: at 1.25 M NaCl for *S. anulatus* J13; at 1 M NaCl for *S. ambofaciens* J27, *Streptomyces thinghirensis* J4, and *S. albidoflavus* H12; at 0.75 M for *S. iakyrus* G10; at 0.5 M for *S. xantholiticus* G22 and *S. xantholiticus* K12.

Table 1. Concentrations of phosphorus (µg PO_4^{3-} mL^{-1}) solubilized by the actinomycetes strains under different NaCl concentrations.

Strain	0 M	0.25 M	0.5 M	0.75 M	1 M	1.25 M	1.5 M
D14	24.8 ^{aA}	12.1 ^{cB}	9.1 ^{cCD}	9.1 ^{eEF}	12.7 ^{cB}	17.3 ^{bA}	10.4 ^{dCD}
G10	17.2 ^{aF}	10.4 ^{cCD}	12.8 ^{bB}	10.1 ^{cCDE}	10.7 ^{cD}	7.1 ^{dG}	10.5 ^{cCD}
G22	23.3 ^{aB}	14.5 ^{bA}	14.4 ^{bA}	11.3 ^{cBC}	10.6 ^{cdDE}	12.1 ^{cB}	9.1 ^{dEF}
G33	10.8 ^{cL}	9.0 ^{dEF}	12.4 ^{dEF}	9.7 ^{cdDE}	9.1 ^{dF}	9.9 ^{cdDE}	14.4 ^{aA}
H12	18.8 ^{aE}	9.9 ^{dDE}	13.3 ^{dDE}	13.2 ^{bA}	11.3 ^{cCD}	9.9 ^{dDE}	9.2 ^{dEF}
H14	22.2 ^{aC}	10.7 ^{cCD}	13.0 ^{cCD}	8.5 ^{dF}	6.6 ^{eG}	6.3 ^{eG}	11.4 ^{cC}
J4	12.1 ^{bcK}	11.4 ^{cBC}	7.9 ^{cBC}	12.9 ^{bA}	16.5 ^{aA}	8.4 ^{eF}	10.1 ^{dDE}
J13	9.9 ^{deM}	15.0 ^{aA}	11.9 ^{aA}	9.5 ^{deEF}	9.3 ^{eEF}	10.8 ^{cdBCD}	12.8 ^{bB}
J21	19.3 ^{aD}	4.6 ^{fG}	8.5 ^{fG}	6.0 ^{eG}	9.1 ^{cF}	11.5 ^{bBC}	8.0 ^{dFG}
J27	12.6 ^{aJ}	4.8 ^{dG}	10.0 ^{dG}	10.7 ^{bCD}	11.1 ^{bCD}	11.0 ^{bBCD}	7.3 ^{cGH}
K12	10.8 ^{cL}	13.8 ^{aA}	12.3 ^{bB}	10.0 ^{cDE}	12.3 ^{bBC}	11.2 ^{bcBC}	10.0 ^{cDE}
K23	14.8 ^{aG}	8.4 ^{eF}	9.9 ^{cDC}	12.1 ^{bAB}	10.5 ^{cDE}	10.0 ^{cdDE}	9.4 ^{deDE}
S2	12.9 ^{aI}	8.0 ^{dF}	6.8 ^{eE}	12.4 ^{aAB}	7.3 ^{deG}	9.3 ^{cEF}	10.5 ^{bCD}
T45	13.9 ^{aH}	11.5 ^{bcBC}	12.2 ^{bB}	11.3 ^{bcBC}	13.3 ^{aB}	10.7 ^{cCD}	6.5 ^{dH}

Results followed by different case letters are not significantly different according to Tukey's post-hoc test ($p < 0.05$) (n = 3). Lower-case letters refer to the comparison of results obtained per strain at different NaCl concentrations (same row). Upper-case letters refer to the comparison of results obtained by the fourteen strains per NaCl concentrations (same column).

Table 2. Concentrations of indole acetic acid (IAA) (µg mL^{-1}) produced by actinomycetes strains under different salt concentrations.

Strain	0 M	0.25 M	0.5 M	0.75 M	1 M	1.25 M	1.5 M
D14	10.7 ^{aD}	4.8 ^{bH}	-	-	-	-	-
G10	12.2 ^{bcC}	11.3 ^{cCD}	13.8 ^{bC}	25.9 ^{aA}	6.1 ^{dE}	6.4 ^{dD}	6.8 ^{d}
G22	12.4 ^{dC}	19.8 ^{bA}	35.6 ^{aA}	18.6 ^{bB}	15.3 ^{cB}	10.7 ^{dB}	-
G33	7.2 ^{F}	-	-	-	-	-	-
H12	-	4.8 ^{cH}	5.1 ^{cF}	8.2 ^{bE}	10.2 ^{aD}	-	-
H14	21.4 ^{aA}	6.9 ^{dFG}	9.7 ^{cE}	12.8 ^{bD}	-	-	-
J4	-	9.8 ^{bcDE}	8.7 ^{cE}	10.2 ^{bE}	12.8 ^{aC}	-	-
J13	9.5 ^{deE}	8.3 ^{cEF}	11.6 ^{dD}	13.9 ^{cCD}	17.6 ^{bA}	25.4 ^{aA}	-
J21	-	6.2 ^{GH}	-	-	-	-	-
J27	9.8 ^{cE}	10.2 ^{cCD}	6.4 ^{dF}	15.2 ^{bC}	18.1 ^{aA}	-	-
K12	7.6 ^{cF}	11.7 ^{bC}	15.2 ^{aBC}	8.2 ^{cE}	14.7 ^{aB}	-	-
K23	-	-	-	-	-	-	-
S2	-	-	-	-	-	-	-
T45	14.8 ^{abB}	13.9 ^{abB}	15.8 ^{aB}	6.0 ^{cF}	12.9 ^{bC}	8.2 ^{cC}	-

Results followed by different case letters are not significantly different according to Tukey's post-hoc test ($p < 0.05$) (n = 3). Lower-case letters refer to the comparison of the results obtained per strain at different NaCl concentrations (same row). Upper-case letters refer to the comparison of the results obtained by the fourteen strains per NaCl concentrations (same column).

3.5. Estimation of 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase Activity

The results for ACC deaminase activity are shown in Figure 2. Among the fourteen isolates, 12 strains had ACC deaminase activity. Higher activity was observed in *S. thinghirensis* J4, *N. dassonvillei* subsp. *dassonvillei* D14, and *S. ambofaciens* J27. No activity was detected in *N. aegyptica* (H14) and *S. thinghirensis* K23.

3.6. Greenhouse Experiment on *T. durum*

During the greenhouse experiment, the bacteria improved plant tolerance to salt (Table 3). The uninoculated plants (no PGPB/with salt stress) were not able to grow at NaCl concentrations $\geq$ 0.5 M. Inoculation with strains *S. iakyrus* G10, *S. ambofaciens* J27, and *S. xantholiticus* K12 allowed plants to tolerate NaCl up to a concentration of 1 M. At higher salt concentrations (1.25 and 1.5 M), no growth was recorded. The response of the inoculated plants to the increase of salt concentration varied according to the species and strain inoculated. At 0.25 M NaCl, the best tolerance was obtained in plants inoculated with *S. xantholiticus* G22 and *N. aegyptica* S2.

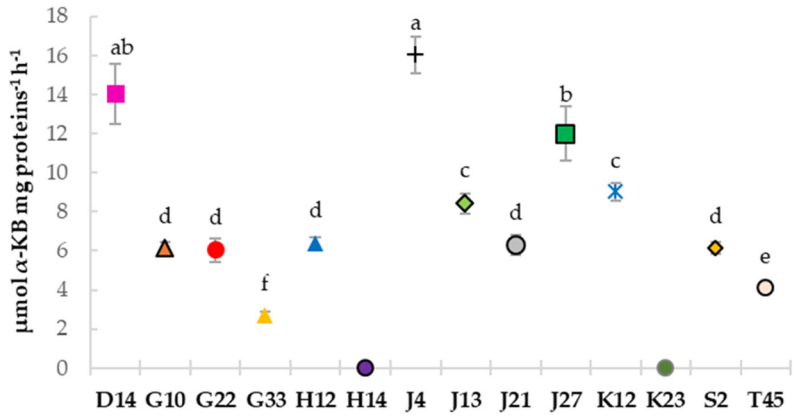

Figure 2. 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (µmol α-KB mg proteins^{-1} h^{-1}) detected in the actinomycetes isolates. Results followed by different case letters are not significantly different according to Tukey's post-hoc test ($p < 0.05$) (n = 3).

Table 3. Survival rate (%) recorded during the greenhouse experiment on *T. durum* for inoculated plant growth-promoting bacteria (PGPB) and uninoculated plants (CNT).

NaCl (M L^{-1})	Survival Rate (%)	
	PGPB	CNT
0	100	100
0.25	100	100
0.5	100	-
0.75	100	-
1	21	-
1.25	-	-
1.5	-	-

Shoots and roots lengths measured after 40 DAS in inoculated plants (D14 and T45) and uninoculated plants (CNT) grown under different levels of salinity are shown in Figure 3. Statistical comparison of data is presented in Figure S1 of Supplementary Materials.

Shoots and roots length was improved in plants inoculated with actinomycetes strains and not subjected to saline stress ($p < 0.05$, Figure S1A). Compared to the uninoculated plants (without PGPB/no salt stress), the lengths of the shoot and root showed an increase of up to 41% (*N. alba* J21) and up to 98% (inoculated with *N. dassonvillei* subsp. *dassonvillei* D14 and *S. anulatus* J13, respectively. The biostimulating effects were negatively affected by the presence of the salt. Best growth was recorded for *N. aegyptica* S2 (+218%) and *S. xantholiticus* G22 (+151%) in terms of shoot and root length compared to the stressed control (no PGPB/with salt stress) ($p < 0.05$, Figure S1B). At 0.5 M NaCl, the maximum shoot length was observed in plants inoculated with *N. alba* (J21), while, the maximum root length was observed in plants inoculated with *N. dassonvillei* subsp. *dassonvillei* D14, and *N. alba* J21 ($p < 0.05$, Figure S1C). At 0.75 M NaCl, plants inoculated with strains *S. iakyrus* G10, *S. xantholiticus* G22, *S. xantholiticus* G33, *S. xantholiticus* K12, and *S. thinghirensis* K23 recorded best shoot length ($p < 0.05$, Figure S1D); *S. iakyrus* G10, *S. xantholiticus* G22, and *S.*

thinghirensis K23, together with those inoculated with *N. aegyptica* H14, *S. anulatus* J13, *N. alba* J21, also recorded best root lengths ($p < 0.05$). At 1 M NaCl, *S. iakyrus* G10 recorded both the best shoot and root lengths ($p < 0.05$, Figure S1E).

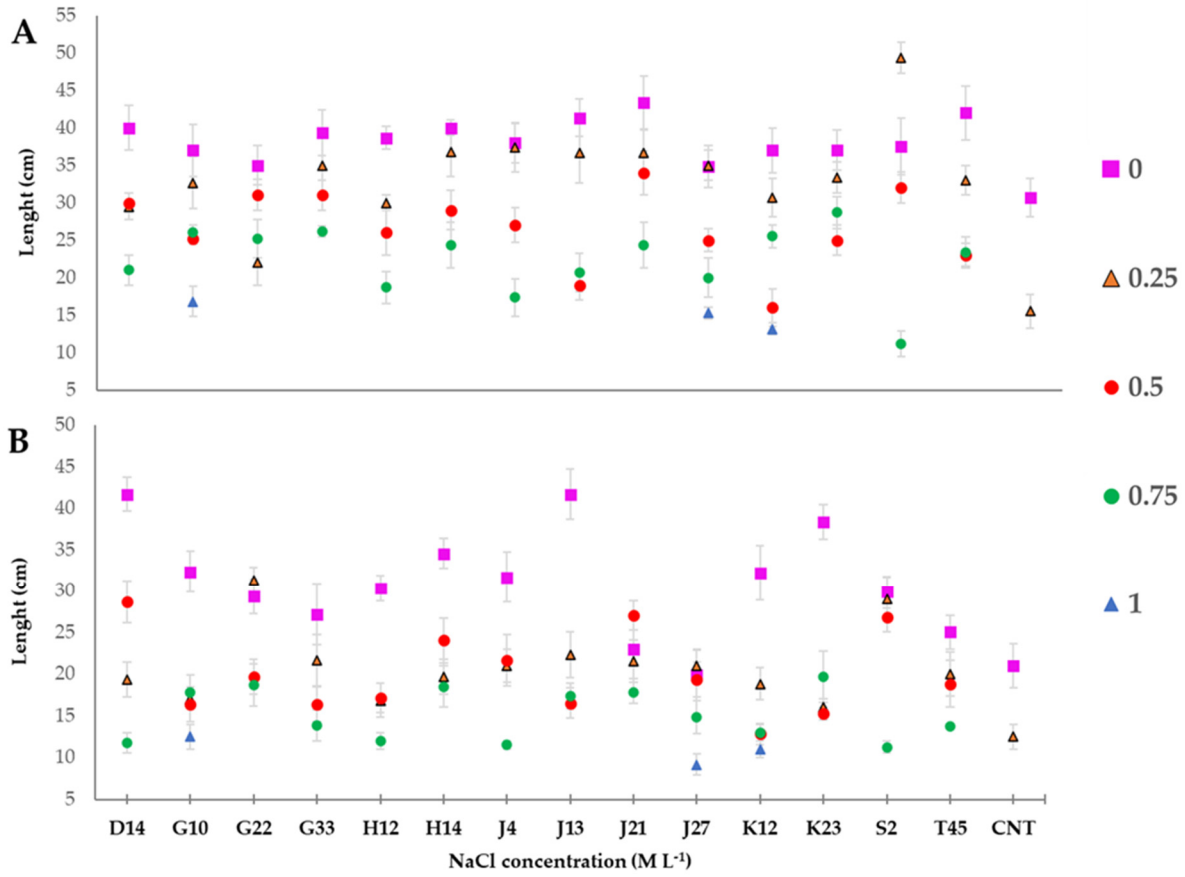

Figure 3. Shoot (**A**) and root (**B**) length of inoculated and uninoculated plants grown without (0 M NaCl) and with NaCl in the soils (0.25 M, 0.5 M, 0.75 M, 1 M NaCl) (n = 3).

Similar trends were recorded for AFDW of shoots and roots. The results of AFDW of shoots and roots obtained after 40 DAS for inoculated plants (D14–T45) and uninoculated plants (CNT) cultivated under different levels of NaCl are shown in Figure 4. Statistical comparison of data is in Figure S2 of Supplementary Materials. In the absence of salt stress, roots AFDW was significantly improved by the presence of the bacteria ($p < 0.05$, Figure S2A). Best values were recorded for strains *S. xantholiticus* K12, *S. ambofaciens* J27, and *N. aegyptica* S2, with a 32% increase on average compared to the uninoculated plants (no PGPB/no salt stress). Plant tolerance over 0.5 M NaCl—promoted by the inoculation—was different according to the actinomycete used. At 0.25 M NaCl, the highest AFDW increases were recorded for *S. albidoflavus* H12, *S. ambofaciens* J27, and *N. aegyptica* S2 for shoots (+24% on average), and *N. dassonvillei* subsp. *dassonvillei* T45 (+14%) for the roots ($p < 0.05$, Figure S2B) compared to the stressed control (without PGPB/with salt stress). At 0.5 M NaCl, the best shoot length was observed in plants inoculated with *N. aegyptica* S2; while, the best root length in plants inoculated with strain *S. thinghirensis* J4 ($p < 0.05$, Figure S2C). At 0.75 M NaCl, the greatest root length was observed in plants inoculated with strain *S. xantholiticus* G33 while plants inoculated with *S. thinghirensis* K23 recorded the greatest root length ($p < 0.05$, Figure S2D). At 1 M NaCl, *S. iakyrus* G10 recorded the highest shoot AFDW ($p < 0.05$, Figure S2E); while, no statistically significant differences were recorded among *S. iakyrus* G10, *S. ambofaciens* J27, and *S. xantholiticus* K12 in terms of root AFDW ($p > 0.05$, Figure S2E).

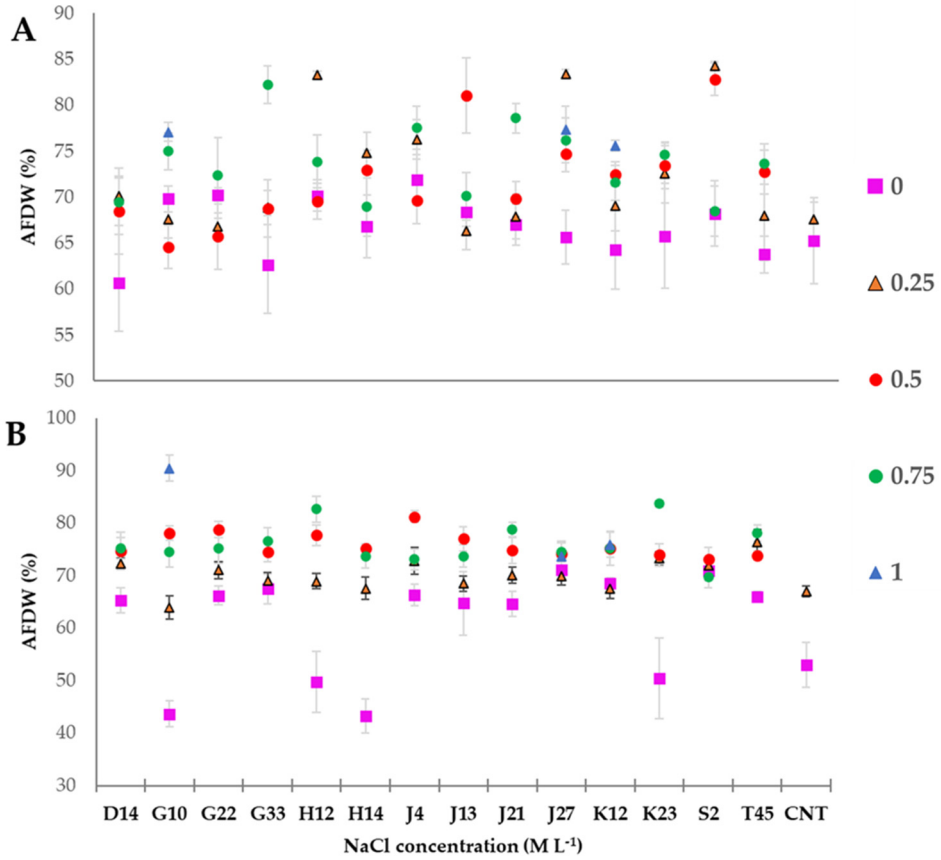

Figure 4. Shoot (**A**) and roots (**B**) ash-free dry weight (AFDW) percentage (%) of inoculated and uninoculated plants with the different actinomycetes strains without (0 M NaCl) and with NaCl in the soils (0.25 M, 0.5 M, 0.75 M, 1 M NaCl) (n = 3).

In the absence of inoculation and without salt stress, we observed normal plant growth; however, the addition of NaCl negatively affect the development of the plant, where growth was stopped at 0.5 M of NaCl addition.

Inoculation with actinomycetes strains also improved chlorophylls content of leaves compared to the uninoculated plants (no PGPB/no salt stress). The concentrations of total chlorophylls (Chl_{tot}) in leaves of plants inoculated with strains D14 and T45 and uninoculated (CNT), at different levels of NaCl, are shown in Figure 5. Chlorophyll a and b concentrations and statistical comparison of data are in Figure S3 of Supplementary Materials.

In the absence of salt stress, the highest Chl_a contents were obtained in samples inoculated with *S. xantholiticus* G22, *N. aegyptica* H14, *S. thinghirensis* K23, and *N. dassonvillei* subsp. *dassonvillei* T45 ($p < 0.05$, Figure S3A). *S. xantholiticus* G22 and *N. dassonvillei* subsp. *dassonvillei* T45 also improved Chl_b content, thus obtaining the best Chl_{tot} ($p < 0.05$, Figure S3A). The induction of halo stress negatively affected the chlorophyll contents. Again, the best results obtained at different NaCl concentrations depended on the strain used for inoculation. For Chl_a, the highest contents were obtained by inoculating *N. dassonvillei* subsp. *dassonvillei* D14/*S. iakyrus* G10/*S. albidoflavus* H12, *N. dassonvillei* subsp. *dassonvillei* D14, and *N. aegyptica* H14 at 0.25, 0.5, and 0.75 M NaCl, respectively ($p < 0.05$, Figure S3B–D). For Chl_b, the highest contents were obtained by *N. dassonvillei* subsp. *dassonvillei* D14/*S. iakyrus* G10/*S. albidoflavus* H12, *N. dassonvillei* subsp. *dassonvillei* D14, and *S. iakyrus* G10 at 0.25, 0.5, and 0.7 M NaCl, respectively ($p < 0.05$, Figure S3B–D). For Chl_{tot}, the highest contents were obtained with inoculation of *S. albidoflavus* H12, *N. dassonvillei* subsp. *dassonvillei* D14, and *S. iakyrus* G10/*N. aegyptica* H14 at 0.25, 0.5, and 0.7 M NaCl, respectively ($p < 0.05$, Figure S3B–D). At 1 M NaCl, *S. ambofaciens* J27 recorded

highest Chl_a contents ($p < 0.05$, Figure S3E), while *S. iakyrus* G10 recorded higher contents of both Chl_a and tot ($p < 0.05$, Figure S3E).

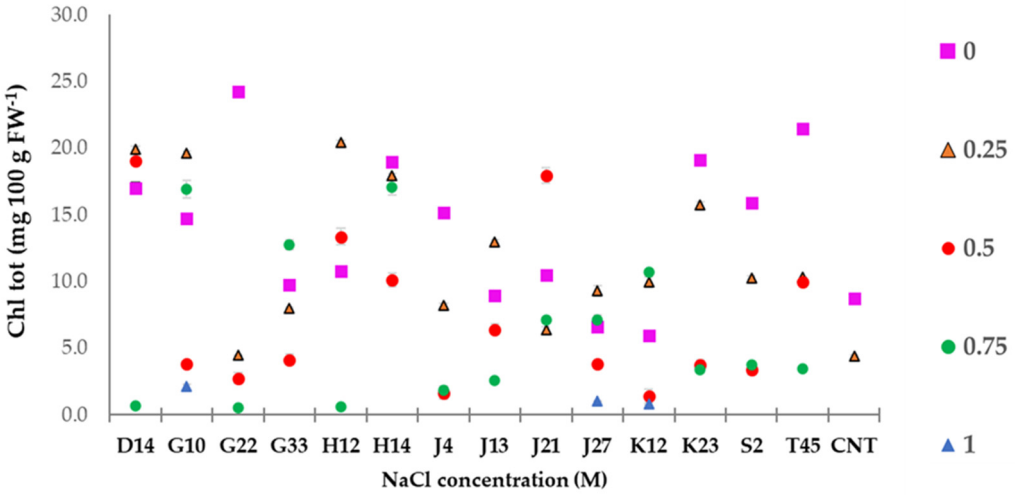

Figure 5. Contents of total chlorophylls (Chl_{tot}) estimated in leaves of inoculated and uninoculated plants without (0 M NaCl) and with salt stress induction (0.25 M, 0.5 M, 0.75 M, 1 M NaCl) (n = 3).

Halo stress significantly affected the ratios between Chl_a and Chl_b in plants inoculated with almost all strains. Chl_a/Chl_b ratios are in Table 4. With increasing NaCl concentrations up to 0.75 M, the chlorophylls ratios in plants inoculated with *N. dassonvillei* subsp. *dassonvillei* D14, *N. aegyptica* H14, *N. alba* J21, and *N. aegyptica* S2 remained the same ($p > 0.05$). For the other strains, higher ratios at higher salt concentrations were observed ($p < 0.05$). This trend indicates good salt stress tolerance. At 1M, the ratios in plants inoculated with *S. iakyrus* G10, *S. ambofaciens* J27, and *S. xantholiticus* K12 decreased ($p < 0.05$). Among these strains, the lowest decrease was observed by *S. xantholiticus* K12, indicating the best tolerance to salt stress.

Table 4. Chlorophyll a/b ratio of inoculated and uninoculated plants without (0 M NaCl) and with salt stress induction (0.25 M, 0.5 M, 0.75 M, 1 M NaCl) (n = 3).

	0 M	0.25 M	0.5 M	0.75 M	1 M
D14	1.9 ^{a}	1.9 ^{a}	1.9 ^{a}	2.1 ^{a}	-
G10	1.9 ^{bc}	2.0 ^{ab}	2.2 ^{a}	1.6 ^{c}	0.3 ^{d}
G22	1.9 ^{b}	2.4 ^{a}	2.2 ^{a}	0.5 ^{c}	-
G33	2.3 ^{a}	1.9 ^{b}	2.2 ^{ab}	2.1 ^{ab}	-
H12	1.8 ^{b}	2.0 ^{b}	1.8 ^{b}	7.7 ^{a}	-
H14	1.9 ^{a}	2.1 ^{a}	2.1 ^{a}	2.2 ^{a}	-
J4	2.0 ^{c}	2.2 ^{c}	3.5 ^{b}	4.1 ^{a}	-
J13	2.3 ^{a}	1.0 ^{b}	2.2 ^{a}	2.0 ^{a}	-
J21	2.1 ^{a}	2.1 ^{a}	1.8 ^{a}	1.5 ^{a}	-
J27	5.5 ^{a}	1.8 ^{b}	1.6 ^{b}	2.3 ^{b}	1.5 ^{b}
K12	2.2 ^{a}	1.9 ^{ab}	0.2 ^{c}	2.1 ^{a}	1.7 ^{b}
K23	2.0 ^{b}	1.9 ^{b}	2.2 ^{b}	3.2 ^{a}	-
S2	2.0 ^{a}	2.0 ^{a}	2.2 ^{a}	1.8 ^{a}	-
T45	1.3 ^{b}	2.3 ^{a}	2.3 ^{a}	2.1 ^{a}	-
CNT	2.5 ^{b}	2.8 ^{a}	-	-	-

Results followed by different lower case letters are not significantly different according to Tukey's post-hoc test ($p < 0.05$) (n = 3).

The results of proline accumulation in plants obtained after 40 DAS inoculated with strains D14 and T45, and uninoculated ones (CNT) are presented in Figure 6. Statistical comparison of data is in Figure S4 of Supplementary Materials.

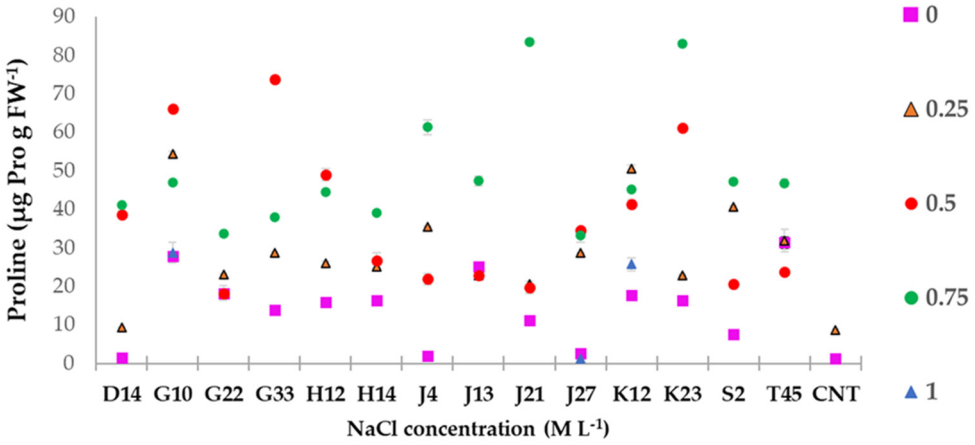

Figure 6. Proline content estimated in shoots of inoculated and uninoculated plants without (0 M NaCl) and with salt stress induction (0.25 M, 0.5 M, 0.75 M, 1 M NaCl) (n = 3).

Without salt stress, there is an accumulation of proline in plant shoots. The inoculated plants have a higher proline value than the uninoculated ones (no PGPB/no salt stress), with variable accumulations depending on the strain ($p < 0.05$, Figure S4A). Salt stress caused a significant proline increase in plants. Inoculated plants had a higher accumulation of proline in response to the increasing NaCl concentrations. At 0.25 M NaCl, the highest proline contents were recorded in plants inoculated with strains *S. iakyrus* G10, *S. xantholiticus* K12, and *N. aegyptica* S2 with an average of 49 µg.g^{-1} FW (+ 460%, $p < 0.05$, Figure S4B) compared to the stressed control (no PGPB/salt stress). At higher NaCl, the proline concentration gradually increased in all inoculated plants. At 0.5 M NaCl, plants inoculated with *S. iakyrus* G10 and G33 accumulated 70 µg Pro g FW^{-1} ($p < 0.05$, Figure S4C). The highest proline accumulation was recorded at 0.75 M (83 µg Pro g^{-1} FW) in plants inoculated with *N. alba* J21 and *S. thinghirensis* K23 ($p < 0.05$, Figure S4D). For strains *S. iakyrus* G10, *S. ambofaciens* J27, and *S. xantholiticus* K12—the only ones that induced plant halotolerance up to 1 M NaCl—moderated amounts of proline were detected (<30 µg Pro g^{-1} FW). The lowest values were obtained for *S. ambofaciens* J27 ($p < 0.05$, Figure S4E).

To explore potential correlations between the outcomes of in vitro and in planta experiments, the data were processed by the Principal component analysis (PCA). Figure 7 shows the PCA biplot obtained from the 0 M NaCl L^{-1} condition. According to the correlations, shoot length, AFDW, and proline accumulation were associated with the production of NH_3 and HCN, root length and chlorophylls with IAA production and phosphate solubilization, and root AFDW with ACC deaminase activity. These associations under salt stress conditions changed (Figure 8) and at 1 M NaCl L^{-1}, the ACC deaminase activity, IAA production, and phosphate solubilization had a good association with proline accumulation and salt stress tolerance (chlorophylls and roots and shoots length and AFDW). The different correlations between in vitro and in planta results demonstrate that the in vitro approach could be useful for the selection of isolates with biostimulating and halotolerant traits.

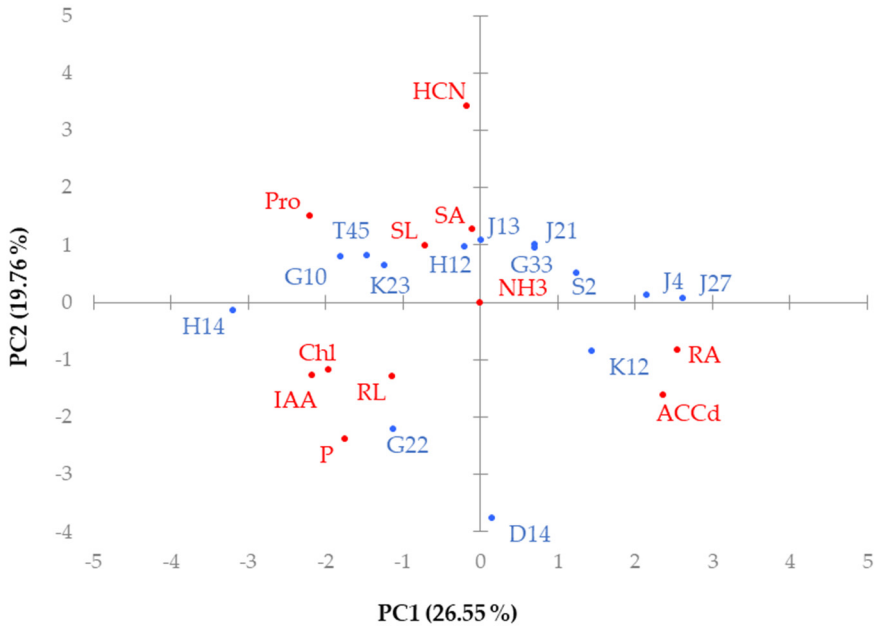

Figure 7. Biplot obtained from the principal component analysis (PCA) on the dataset of in vitro and in planta results (loadings, in red) recorded for actinomycete strains (scores, in blue) without salt stress. In the figure: ACCd, 1-aminocyclopropane-1-carboxylate deaminase activity; Chl, total chlorophyll; SL, length of shoots; RL, length of roots; RA, ash-free dry weight of roots; SA, ash-free dry weight of shoots; Pro, proline; P, phosphate solubilization; IAA, indole-3-acetic acid.

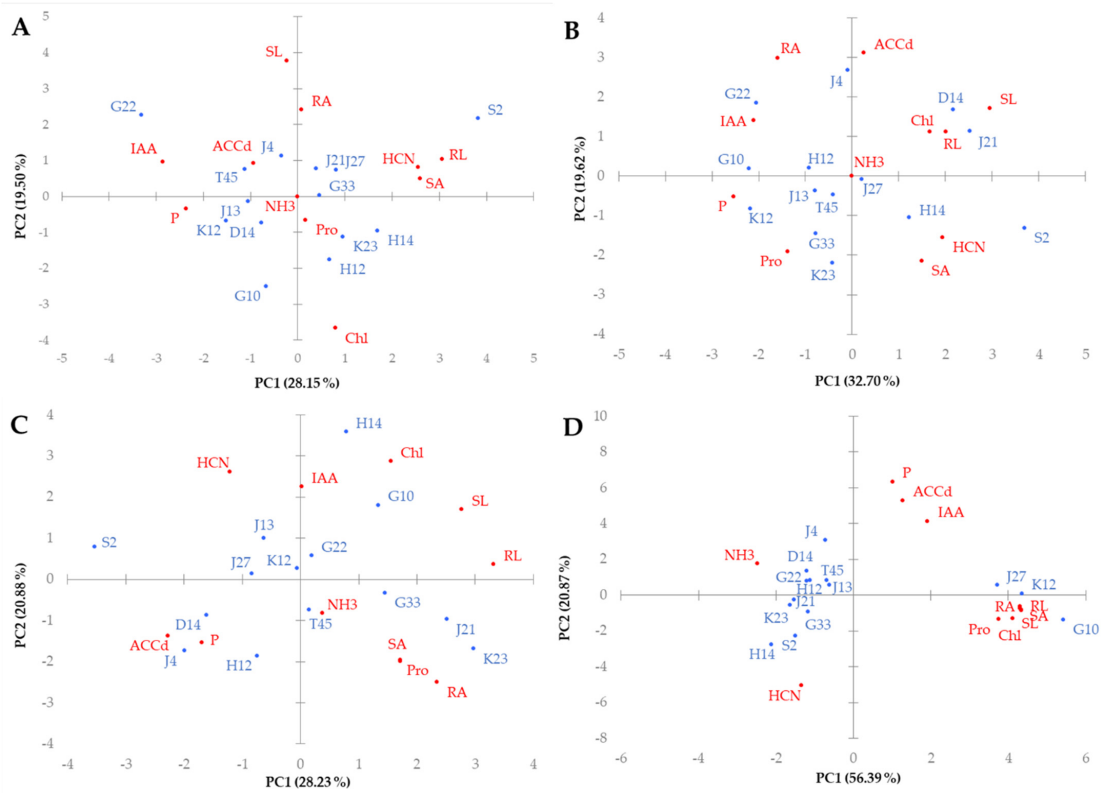

Figure 8. Biplot obtained from the principal component analysis (PCA) on the dataset of in vitro and in planta results (loadings, in red) recorded for actinomycete strains (scores, in blue) under different salt stress concentrations ((**A**)—0.25 M NaCl L^{-1}, (**B**)—0.5 M NaCl L^{-1}, (**C**)—0.75 M NaCl L^{-1}, and (**D**)—1 M NaCl L^{-1}). In the figure: ACCd, 1-aminocyclopropane-1-carboxylate deaminase activity; Chl, total chlorophyll; SL, length of shoots; RL, length of roots; RA, ash-free dry weight of roots; SA, ash-free dry weight of shoots; Pro, proline; P, phosphate solubilization; IAA, indole-3-acetic acid.

4. Discussion

Plant inoculation with halotolerant bacteria is one the most sustainable way to cope with the numerous deleterious effects of salinity [50–53]. Several microorganisms have been reported as promoters of plant growth under saline stress [12,16,54]. Halotolerant/halophilic PGP bacteria are physiologically adapted to environmental changes and increase plant tolerance to salinity thanks to several PGP traits [52]. In the present work, we proposed the use of actinomycetes isolates to induce salt stress tolerance in wheat. Halotolerant actinomycetes with PGP traits are already recognized as inducers of salt tolerance [55]. However, studies on their application as halotolerant agents are still scarce.

Among PGP traits useful for the induction of tolerance in plants, phosphate solubilization is extremely important for the growth and yield of crops [26,56–60]. Salinity decreases the low available content of phosphorous [58] and limits its absorption by the plant root [61]. Phosphate solubilizing bacteria (PSB) can convert insoluble phosphorus to soluble (PO_4^{3-}) and available (e.g., HPO_4^{2-}, H_4PO^{4-}) [62].

A decrease in phosphate solubilization rate in a saline environment is reported [61,63]. In our case, the solubilization capability was negatively affected by the presence of NaCl in almost all strains. However, a good solubilization ability has been kept, possibly due to the origin of these strains isolated from saline soils. Our findings show that generally, the maximum solubilization rate occurred in the presence of a certain amount of NaCl, depending on the strain, and lower without NaCl. These findings are in accordance with previous studies; many bacteria require NaCl for better solubilization of inorganic phosphate [64,65] and microorganisms originating from saline soils are a valid tool to improve the availability of phosphorus in soil and improve wheat growth parameters [66].

Another mechanism used by PGPB to induce tolerance in plants against various environmental stresses is the regulation of phytohormones synthesis [67,68]. Phytohormones help plants tolerate salt stress by developing a protective response against stress, promote cell proliferation in the root system and increase the surface area for water and nutrient uptake through the overproduction of root hairs [50,69]. Among phytohormones, IAA directly influences plant growth, improving nutrient uptake, and plant health under stress conditions [70,71]. Growth regulators, such as auxins, are known to reduce salinity-induced dormancy in wheat seeds [72]. Many PGPB can produce IAA and participate in plant growth and development [73]. Salt has been shown to inhibit plant growth, phytohormones production, and interfere with important cellular processes [74]. The decrease in hormone levels in the plant's root system results in a reduction in growth and development [75]. Salinity does not affect auxins production and physiological processes in plants treated by salt-tolerant PGPB [67]. As for our strains *N. dassonvillei* subsp. *dassonvillei* D14, *N. aegyptica* H14, and *S. xantholiticus* G33, several authors reported that the presence of salt stress may cause a decrease in IAA synthesis [76–79]. According to the literature [68], an improvement on the synthesis of this hormone was found in the presence of different salt concentrations in the case of strains *S. iakyrus* G10, *S. xantholiticus* G22, *S. anulatus* J13, *S. ambofaciens* J27, *S. xantholiticus* K12, *N. dassonvillei subsp. dassonvillei* T45.

Crops subjected to saline stress may be more susceptible to phytopathogen attacks, PGPB can provide volatile compounds useful for plant protection, such as HCN and NH_3 [80,81]. These compounds are involved in various biochemical and physiological processes, including cell signaling and plant growth improvement [82]. HCN disrupts pathogenic cells by inhibiting electrons transport, causing cell death [81]. The presence of adequate amounts of NH_3 increases plants' biomass and resistance, by inducing root branching and elongation [80]. The ability to produce these volatile compounds was shown by nearly all our actinomycetes isolates not exposed to salt stress. In the presence of increasing concentrations of salt, only strain *N. aegyptica* H14 retained both synthesis abilities up to 1.25 M NaCl. The production of these compounds is important for plant development, particularly under stress conditions. Under salt stress, the need for nutrients for metabolic activities increases and the presence of volatile compounds can meet this demand [83].

Substances that inhibit growth also endanger the plant physiological status under salt stress conditions. Among them, ethylene is a gaseous hormone produced by most plants [84]. In normal conditions, it is involved in plant growth and development. Its release is regulated by environmental changes [17] and its levels increases during stressful conditions [85], becoming harmful for plants [42,85]. The ACC deaminase present in PGPB promotes the regulation of the ethylene levels, protecting plants against harmful increases [80,86]. This trait, present in almost our actinomycetes, provides a great advantage for crop's sustainable development in stressful conditions [87]. Different studies underlined that fertilization with PGPB possessing ACC deaminase promotes plant growth development [86,88] and provides plant protection against various environmental stresses [89].

In light of the PGP traits mentioned above, the improvement of plant growth under halo stress could be the result of an improvement of nutrients uptake, hormones regulation [90,91], and enzymatic activities [92]. Salinity negatively affects plant growth and yield [93,94] with a high negative impact on the root system [51]. The presence of NaCl affects also aerial parts, with a reduction in photosynthesis due to a decrease of chlorophylls content [7,95,96]. Our findings underlined a good influence of actinomycetes inoculation on plants. In particular, the inoculation with strains *S. iakyrus* G10, *S. ambofaciens* J27, and *S. xantholiticus* K12 induced a plant salinity tolerance up to 1 M NaCl. The nature of halotolerant actinomycetes used in this study may be the subject of a future in-depth study on their use as biofertilizers to improve crops in soils affected by salinity. Biofertilization strategy already showed positive outcomes in plant growth and development of several crops [67,97,98]. The accumulation of proline is an important mechanism for osmotic regulation under salt stress. Our results showed that at increasing NaCl concentrations, the accumulation of proline is more pronounced in plants inoculated by actinomycetes strains. In response to environmental stresses, plants store proline and other compatible solutes as an adaptive response to osmotic unbalance. In fact, proline promotes water retention in the cytoplasm, at high salt concentrations; it appears to be a mechanism for stress tolerance in plants. Proline is an osmolyte stabilizing the cells and a source of nitrogen, carbon, and energy for plant growth. This amino acid provide also tolerance to salt stress by cell turgor maintenance and cells protection against oxidative damages [4,5,99–103].

5. Conclusions

Salinity is currently one of the main problems of agricultural soil with a strong negative impact on crops productivity. To overcome this problem only a few effective and sustainable actions have been proposed. Therefore, studies on sustainable strategies to overcome soil salinity should be encouraged. A promising tool for improving crop productivity under salt stress conditions is inoculation with halophilic/halotolerant PGPB.

The present study allowed to select PGPB based on their ability to induce salt stress tolerance in plants up to 1 M NaCl. Among fourteen isolates of actinomycetes, the best induction of tolerance was recorded for plants inoculated with strain *S. iakyrus* G10, *S. ambofaciens* J27. *S. xantholiticus* K12 also showed an interesting tolerance induction at 1M NaCl. Further studies should be undertaken to evaluate the possibility of producing biofertilizers with these strains. Moreover, these strains could be joined in a consortium, to evaluate possible synergistic effects. The effectiveness of the formulations should be also investigated in open field experiments and on different crops. Even if it is necessary to make further investigation, the results obtained in the present work are a good starting point for the development of biofertilizers useful for overcoming the high salinity in soils.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/soilsystems5020026/s1, Figure S1: Comparison of shoot and root length obtained for the inoculated (D14-T45) and uninoculated plants (CNT) without salt stress induction (A—0 M NaCl L^{-1}) and under different salt stress concentrations (B—0.25 M NaCl L^{-1}; C—0.5 M NaCl L^{-1}; D—0.75 M NaCl L^{-1}; E—1 M NaCl L^{-1}) (n = 3), Figure S2: Comparison of shoot and root ash-free dry weight (AFDW) obtained for the inoculated (D14-T45) and uninoculated plants (CNT) without salt stress

induction (A—0 M NaCl L^{-1}) and under different salt stress concentrations (B—0.25 M NaCl L^{-1}; C—0.5 M NaCl L^{-1}; D—0.5 M NaCl L^{-1}; E—0.75 M NaCl L^{-1}; F—1 M NaCl L^{-1}) (n = 3), Figure S3: Comparison of Chlorophylls a, b and total (Chl a, b and tot) obtained for the inoculated (D14-T45) and uninoculated plants (CNT) without salt stress induction (A—0 M NaCl L^{-1}) and under different salt stress concentrations (B—0.25 M NaCl L^{-1}; C—0.5 M NaCl L^{-1}; D—0.5 M NaCl L^{-1}; E—0.75 M NaCl L^{-1}; F—1 M NaCl L^{-1}) (n = 3), Figure S4: Comparison of proline accumulation obtained for the inoculated (D14-T45) and uninoculated plants (CNT) without salt stress induction (A—0 M NaCl L^{-1}) and under different salt stress concentrations (B—0.25 M NaCl L^{-1}; C—0.5 M NaCl L^{-1}; D—0.5 M NaCl L^{-1}; E—0.75 M NaCl L^{-1}; F—1 M NaCl L^{-1}) (n = 3).

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